ROOT DYNAMICS AND CARBON ACCUMULATION OF SIX WILLOW CLONES IN SASKATCHEWAN

A Thesis Submitted to the College of Graduate Studies and Research

In Partial Fulfillment of the Requirements for

The Degree of Master's of Science

In the Department of Soil Science

University of Saskatchewan

Saskatoon, Canada

By

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ABSTRACT

Short rotation woody crops have gained global interest as an alternative energy source to fossil fuels. The availability of this resource is, however, dependent on successful research trials and the identification and quantification of the environmental controls on rapid growth. Knowledge of willow root dynamics is required to determine belowground and aboveground growth relationships, and to provide valuable inputs for the development of a willow carbon model. The objectives of this study were to 1) determine fine root turnover, biomass, and productivity of six willow clones over two growing seasons at four locations in Saskatchewan using the minirhizotron method; 2) determine fine root biomass and fine root carbon sequestration of six willow clones over one growing season at four locations in Saskatchewan using the soil coring method; and 3) determine lateral coarse root structure of six willow clones at two locations in Saskatchewan.

Monthly fine root biomass and production was estimated for six willow clones in Saskatoon, Saskatchewan using repeated minirhizotron observations from May to September of 2008 and 2009. Fine root biomass increased from 0.78 Mg ha^{-1} in May 2008 to 25.75 Mg ha^{-1} in September 2009. Significant differences were seen between months throughout each growing season, but not between the clones. Mean monthly productivity reached its peak of 8.00 Mg ha^{-1} in July 2009. Mean turnover for all the clones was 0.96 yr^{-1} and mean longevity was 1.04 yr^{-1}. The high fine root biomass estimates determined by the minirhizotron method in Saskatoon suggest that this method is not suitable for use in a Vertisolic soil. There was no significant effect of clone on fine root productivity, biomass, turnover or longevity ($P < 0.05$).

Fine root biomass estimates from the soil cores were lower than those from the minirhizotron method. The mean fine root biomass value in Saskatoon for September 2008 was 0.298 Mg ha^{-1}. Mean fine root biomass at each site from September 2007 to September 2008 ranged from 0.022 Mg ha^{-1} to 0.915 Mg ha^{-1}. Mean root carbon content ranged from 0.010 to 0.426 Mg C ha^{-1}. Fine root biomass and root carbon content were significantly different between each site, with the exception of Saskatoon and Estevan ($P < 0.05$). Differences in fine root estimates between the sites are suggested to be a function of the soil texture and moisture accessibility at each site. This research indicates that willow roots in Saskatchewan find initial establishment difficult in low moisture, fine textured soils. Also, approximately 44.6 % of fine root biomass is comprised of C, and decomposes to form soil organic matter. Therefore, fine roots have potential to store substantial amounts of carbon if growing conditions are suitable.

ACKNOWLEDGEMENTS

I am humbly thankful for the guidance and support I received from my supervisor, Dr. Ken Van Rees, whose knowledge and passion for forest research helped foster my interest in the Canadian bioenergy sector. Dr. Van Rees' influence in my life is not limited to science, but also extends into the arts, as many team field trips included days of learning how to appreciate the scientific mysteries of nature as well as the inherent beauty of the landscape. I am very proud to have been a part of "Team Van Rees" for the past two and a half years. I would also like to extend a sincere thank you to my supervising committee, Drs. Angela Bedard-Haughn and Darwin Anderson from the Department of Soil Science, and my external advisor, Dr. John Kort from the Shelterbelt Centre in Indian Head, Saskatchewan. I am also grateful to NSERC Strategic Grant program for funding the project, of which I was a part.

Throughout two field seasons, I received help from a hard working team of people who made the many minirhizotron and soil coring trips possible. I am indebted to Doug Jackson, Sheala Konecsni, Ryan Hangs, Shawn McDonald, Josh Leventhal, Kaitlin Strobbe, Melissa Van Rees, and Rachel Van Rees. I would also like to thank my friends and fellow graduate students in the department for their encouragement and for being of additional assistance to me. I also wish to acknowledge our site collaborators: Pacific Regeneration Technologies Inc., SaskPower Shand Greenhouse, and the Bill Brewster farm.

I wish to thank my family and friends for continuously encouraging me and for taking interest in my project. Specifically, I thank Linda, my mom, for being such a wonderful source of encouragement and wisdom. I also thank James, my best friend, for learning as much as possible about my project and helping me explain the heavy details to friends. Lastly, as a roommate, friend, and coworker, I thank Sheala Konecsni for being a voice that encouraged me to do the best I could. Thanks for dancing with me, Sheala!

DEDICATION

I wish to dedicate this work to those whose love, faith and wisdom throughout the years have taught me so much about life and I am endlessly thankful to them.

Marty and Linda Welden, Ione Surbey, Candace Stadnyk

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Growing degree days ..... GDD

Root collar diameter ..... RCD

Root length density ..... RLD

Short rotation woody crops ..... SRWC

Statistical Analysis Software ..... SAS

1.0 INTRODUCTION

In the past few decades, global concern regarding the environmental state of the planet has grown strong, and the distinct rise in atmospheric CO_2 levels since the start of the industrial era has encouraged a shift in the hierarchy of consumer priorities, placing the health of the environment at the forefront of scientific advancement. Analogous to public concern, many energy companies are eager to investigate renewable energy sources. The lines between energy, environment and economy are blurred as the benefits of alternative energy sources become more apparent. Pertinent research in any of these three areas is likely to positively influence the other two. Thus, environmental research regarding the mitigation of atmospheric CO_2 is integral to ensure the future health of the planet. The research detailed in this thesis is intended to provide more realistic inputs for carbon budget models, which are important for understanding and mitigating changing climate.

As the CO_2 level in the atmosphere increases at a rate of > 2 ppm y^{-1} (Raupach et al., 2007), many aspects of the biosphere are experiencing change, including soil moisture and plant nutrient concentrations (Kruijt et al., 2008; Paterson et al., 2008; Raupach et al., 2007; Zvereva and Kozlov, 2006). Fossil fuel combustion and land use change constitute a majority of the anthropogenic contribution of CO_2 to the atmosphere (Sabine et al., 2004). Strategies to reduce net CO_2 emissions have highlighted the need for alternative technologies and energy sources. Bioenergy crops such as willow reduce CO_2 emissions to the atmosphere because they use C fixed by photosynthesis rather than fossil fuel. Although the CO_2 is returned to the atmosphere during burning, C sequestration in the soil as roots and organic matter results in a net CO_2 reduction in the atmosphere. With its vast geographical expanse, Canada has an advantage in this respect, as it has ample natural resources from many sectors, notably the forestry sector. At 60%, biomass constitutes the largest proportion of electrical generating capacity in Canada when compared to other forms of renewable energy such as wind and small hydro, at 12.7% and 26.7%, respectively (Bradley, 2006).

One mitigation effort has involved the co-firing of biomass with coal to generate electricity. This not only effectively aids in reaching CO_2 emission targets, but also provides an economically efficient use of biomass (Baxter, 2005). The process of co-firing is intentionally congruent with current technologies, as energy producers do not favor a strong deviation from existing applications (Tharakan et al., 2005). Biomass plays a significant role as co-firing material, and substantial efforts have been made to broadly institute this biomass crop into the energy sector. When compared to coal, biomass contains low amounts of ash and nitrogen, and virtually no sulfur (Hughes, 2000). A growing body of research on dedicated short rotation woody crops (SRWC) has reinforced its

environmental and economic importance. With continued efforts, SRWC can advance as a dominant player in the restructuring of conventional energy production.

Salix (willow) is one such SRWC that is currently under investigation for global use as an alternative energy crop because of its high yield potential under optimal conditions. In a study comparing two willow clones on sandy and clay sites in Southern Quebec, Labrecque and Teodorescu (2003) reported the highest willow yield in Canada to date, at 70.36 Mg ha^{-1} at the end of the second cycle of growth. A clone is a genotypically similar stock; a species, a hybrid, or a sub-species. A majority of the research on willow biomass crops has focused on the aboveground portion of the system, as this fraction offers an obvious direct return. Less is known about the root systems of willow plantations due to the difficulty in quantifying them. Knowledge about the belowground portion of the system is crucial in SRWC, as this system relies on high quantities of aboveground regeneration. This study attempts to quantify and examine the dynamics of willow root systems.

The multiple roles of willow root systems amplify the importance of willow as an environmentally beneficial bioenergy crop. Anchorage, nutrient acquisition and storage, and phytoremediation are among the notable benefits (Corseuil and Moreno, 2001; Jackson et al., 1997; Karrenberg et al., 2003). The successful establishment of a willow plantation is highly dependent on the propagation of the root system. Furthermore, willow has the potential to capture substantial amounts of C in its extensive root system, adding to the soil C pool (Block et al., 2006; Lemus and Lal, 2005; Zan et al., 2001). The rate at which this occurs is primarily a product of the rate of fine root turnover in the soil (Block et al., 2006). It is important to understand the site and clone specific root dynamics, as well as optimal environmental conditions for rapid root growth in willow plantations during establishment and for validating willow C budget models.

Willow plantations have not been established in Saskatchewan on an operational basis and it is uncertain how they will perform in relation to our soil and climatic conditions. Examining the root dynamics and potential C sequestration belowground is needed to better understand the agronomy of these systems as well as developing models for C sequestration. It was hypothesized that there would be a significant difference in fine root turnover, biomass and productivity among the six willow clones in Saskatoon using the minirhizotron method, and there would be a significant difference in fine root biomass and C sequestration among the six willow clones and among the four sites using the sequential soil coring method. The null hypothesis states that there would be no significant relationship between fine root turnover, biomass or productivity among the six willow clones or among the four sites. Therefore, the objectives of this study were to:

1. Determine willow fine root biomass, productivity and turnover using the minirhizotron method for six willow clones over two growing seasons in Saskatoon, Saskatchewan.
2. Determine fine root biomass using the sequential soil coring method, and determine the amount of C being sequestered belowground for six willow clones at four locations in Saskatchewan.
3. Determine the coarse root structure of six willow clones at two locations in Saskatchewan using the whole tree excavation method.

This thesis consists of a total of five chapters with Chapter 2 reviewing pertinent literature, including root morphology, root measurement methods, and fine root carbon capture and storage. Chapter 3 determines fine root productivity and mortality of six willow clones in Saskatoon using the minirhizotron system. Chapter 4 discusses the fine root biomass of six willow clones at four locations in Saskatchewan using the soil coring method as well as excavating the entire root systems of willows in Saskatoon and Prince Albert. This chapter also describes the amount of C sequestered belowground. Chapter 5 consists of a general discussion of the studies, emphasizing the relevance of this project and placing it into a Canadian bioenergy perspective.

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2.0 LITERATURE REVIEW

2.1 Overview of Bioenergy

The expanding world population and demand for energy has put pressure on the supply of energy (Kaygusuz, 2002). This increasing demand, however, will not be satisfied using conventional energy sources as oil production is expected to reach its peak and begin to decline over the next 30-50 years (Dorian et al., 2006). The high environmental cost associated with the overuse of fossil fuels has left energy supply companies concerned with environmental protection and has highlighted the urgency for alternative energy sources (Jagadeesh, 2000; Weih, 2004). Biomass has been recognized as a potential source of energy to future sustainable development (Hall, 1997). The current contribution of bioenergy to total global energy use is 13% (Sims, 2001) and current estimates state that biomass from agricultural sources is equivalent to approximately 24% of Canada's annual fossil fuel energy use (Wood and Layzell, 2003). Research in many topics of bioenergy plantations from establishment to harvest is currently underway to help increase the contribution that biomass could make to primary energy use in Canada.

Along with their substitution for fossil fuels, biomass crops can help to mitigate the threat of global climate change through carbon sequestration. The extensive perennial root systems of woody bioenergy crops and their high biomass yield increase the amount of carbon (C) being sequestered when compared to agricultural crops (Lemus and Lal, 2005). In order to create an overall C savings or maintain a neutral C balance, energy expended on land conversion and inputs such as fertilizers to create the biofuel crops must be considered (Fargione et al., 2008). Therefore, it is necessary to seek a bioenergy resource that is capable of establishment on sites with minimal preparation, while allowing for maximum environmental benefits and biomass production.

2.2 Willow as a Source of Bioenergy

Short rotation willow crop systems are currently under investigation because of their ability to fulfill the multiple ecological objectives, and have already been shown to have significant environmental benefits (Rockwood et al., 2004). Willow plantations can remain productive for approximately 20 to 30 years (Abrahamson et al., 1998; Heller et al., 2003) with removal of biomass occurring approximately seven times in 3 to 4 year rotations (Abrahamson et al., 1998; Keoleian and Volk, 2005; Ledin, 1996).

The high output to input ratio for energy is one of the many advantages of growing willow for biofuel. At the end of each harvest (all seven of the 3 to 4 year rotations) approximately 55 units

of harvested bioenergy can be created with 1 unit of fossil fuel input. Yield, however, is highly dependent on many variables including genetic diversity, soil fertility, climate and the spacing arrangement of the plantation (Keoleian and Volk, 2005). Trials in New York have produced yields as high as 27 Mg ha^{-1} yr^{-1} in an irrigated and fertilized plantation (Adegbidi et al., 2001).

2.3 Willow Root Soil Remediation

The ability for willows to efficiently take up nutrients has been harnessed in their use for soil remediation purposes (Mirck et al., 2005; Rockwood et al., 2004). Greger and Landberg (1999) examined the potential of willow for its use in phytoremediation and found that some willow clones have the capacity for both high accumulation and tolerance of heavy metals. Wastewater biosolids that would otherwise be released to the environment have been used in willow plantations as an organic fertilizer (Heller et al., 2003). The absorption and filtering properties of willow are enabled by their extensive perennial root systems as well as their long lifespan compared to annual crops (Keoleian and Volk, 2005; Perttu, 1998). Wang (2004) found that although there was significant variation in the sensitivity to mercury (Hg) among six willow clones, accumulation of Hg in their root systems supports the potential use of willow for phytoremediation. A better understanding of willow root dynamics in Saskatchewan will allow for more accurate inferences to be made regarding remediation practices and techniques using willow in this region.

2.4 Willow Root Systems

Although roots play a critical role in the overall functioning of a plant there is relatively little information about specific root distribution of short-rotation willow crops (SRWC) (Volk et al., 2001). Along with soil remediation, willow root systems are useful for plant stabilization and soil erosion control in riparian areas (Schaff et al., 2002). To ensure maximum economic gain from a willow plantation it is essential to understand the belowground biomass dynamics. This knowledge allows for the selection of the most suitable species for a region based on carbon allocation to the root system, which influences the growth of the stem (Rytter and Rytter, 1998).

2.4.1 Root morphology

The root system of a plant functions as the structural support, and in the case of fine roots, as the conduit through which soil nutrients and water are absorbed (Puttsepp, 2004). Fine roots are defined as those being less than 1 or 2 mm in diameter, and although they may only constitute less than 1% of standing biomass of a mature tree, they can account for approximately 30-50% of annual

net primary production (NPP) (Grier et al., 1981; Rytter, 1999). Willow roots systems are characterized by tap and fibrous root arrangements (Puttsepp, 2004). Brundrett et al. (1990) described the willow root system being comprised of long straight roots bearing shorter, curved roots with root hairs scattered throughout the system. Durall et al. (1994) observed a darkening pattern of the root system that occurs in conjunction with senescence. Hence, root colour can be related to root age.

2.4.2 Root distribution

Willow roots typically reach an average depth of 25-30 cm during the first growing season, extending into greater depths during the second growing season (Rytter and Hansson, 1996). The rooting depth is primarily responsive to the moisture gradient in the soil profile (Volk et al., 2001). In contrast, Horton and Clark (2001) compared willow and saltcedar (*Tamarix chinensis)* survival rates by subjecting them to various soil moisture regimes and found that across all treatments, willow roots tended to have significantly more lateral growth than saltcedar. In a study done by Keller et al. (2003), willow root systems were observed to have relatively deeper root distribution when compared to the annual crops, mustard (*Brassica juncea*), tobacco (*Nicotiana tabacum*) and maize (*Zea mays*). This vertical distribution is likely a factor in the high survival rates of willow plantations established on marginal sites because deeper rooting allows for greater accessibility to limited soil resources (Volk et al. 2001). Keller et al. (2003) suggested that this extension could account for the significant root density of willows. Examination of the horizontal root distribution is more difficult than vertical distribution due to root overlap under the high planting densities of SRWC systems.

2.5 Definition and Importance of Fine Root Systems

The definition of fine roots (< 2 mm diameter) (Pregitzer et al., 2002) is either based on their physiology or function. This non-standardized definition, along with the difficulty of observing fine roots, has led to scarcity on this topic in the literature. Notwithstanding, fine roots are typically defined by their placement in a diameter class. Belowground, the plant system performs many important functions for the plant, depending on the particular part of the root system. While coarse roots function mainly as a support and anchorage system, fine roots function as the nutrient and water conduit for the rest of the plant (Puttsepp, 2004). The fine root system is the portion with the most surface area and highest turnover rate, and in some cases, may surpass aboveground production (Rytter, 1999). The numbers for NPP are widely variable but are generally high. On average, 40% of

annual NPP is allocated to belowground biomass, most of which is in the form of fine roots (Rytter, 2001).

At plant maturity, it is commonly assumed that fine roots are in a constant state of equilibrium in terms of production and mortality, highly responsive to the heterogeneity of the root zone microclimate. Fine roots grow, senesce, and decompose at a very rapid rate in a process that demands large amounts of carbon, and accounts for a large portion of the ecosystems NPP. Rytter et al. (2001) determined annual NPP for a willow plantation to be 6.61 Mg ha^{-1} yr^{-1}. The dynamic nature of the environment sparks interest in how individual plants adapt to changes that are seen in the soil. The roots carry out nutrient exploitation and down-regulation of respiration in a systemic manner to ensure survival in ever-changing conditions (Pregitzer et al., 2002). Currently, fine root production estimates that are derived by common methods are heavily reliant on estimates of fine root turnover, which are limited by considerable inaccuracies in the data. There is a debate in the literature whether fine root production is increased on nutrient rich or nutrient poor sites (Gower and Vitousek, 1989; King et al., 2002; Nadelhoffer et al., 1985).

2.5.1 Root morphology and seasonal temporal dynamics

There are, as of yet, relatively few studies done on the morphological stages of willow fine roots. General assumptions maintain that the coarse roots reach a steady mass and remain stable for the remainder of the lifetime of the crop (Heller et al., 2003), while the temporal dynamics of fine roots are more closely correlated to the surrounding environment (Leuschner et al., 2004). Turnover studies are particularly difficult due to the simultaneous nature of fine root production and mortality (Rytter, 1999; Rytter and Hansson, 1996). In the minirhizotron system, dead roots are those that are classified as black or no longer present in images. In minirhizotron studies, problems have arisen with the discrimination of dead roots, as their occurrence is more of a function of sampling frequency, whereas new roots are more evident in an image (Bernier and Robitaille, 2004).

The processes of fine root production and mortality in a system are difficult to examine due to the high degree of spatial and temporal variability (Norby et al., 2004). However, Rytter (2001) found that the ratio of fine willow root to aboveground production ranges from 0.4 to 1.2, depending on the environment and year of growth.

Production and mortality occur simultaneously and create a constantly changing, dynamic picture of the fine root portion of the system (Aber et al., 1985; Burke and Raynal, 1994; Rytter, 1999). Deeper soil layers are explored by the root system during times when water requirements are

high, resulting in higher productivity in the fine root system. During the winter months in a northern hardwood forest, water requirements are low and fine root productivity decreases (Hendrick and Pregitzer, 1996). As production of fine roots is highly correlated to water and nutrient availability in the soil, the processes contributing to mortality have yet to be fully understood (Wells et al., 2002).

2.5.2 Soil factors affecting root growth

Root growth occurs in response to various heterogeneous soil conditions that will either hinder or encourage exploration throughout the soil, and is highly responsive to the surrounding environment, thriving most in areas of optimal nutrient concentration (Malamy, 2005; Volk et al., 2001). Nitrogen fertilizer application and leaf litter decomposition increase the nutrient concentration of the soil layer near the surface (Rytter and Hansson, 1996). These findings explain why there is a shallow distribution of willow root systems. Concern exists, however, about the removal of nutrients along with harvesting, causing soil nutrient depletion (Adegbidi et al., 2001; Perttu, 1998). Abrahamson et al. (1998) attempted to mitigate nutrient depletion by applying fertilizer while the crop was actively growing to ensure maximum uptake by the crop, as opposed to being lost to the soil or assimilated by the weeds.

The moisture regime of the soil is thought to be a key factor in the establishment and proliferation of an extensive root system. Extreme moisture conditions, such as flooding or drought, may have an adverse effect on willow biomass production by affecting stomatal conductance (Greer et al., 2006). The mechanical impedance of the soil is a factor that is also regarded as a hindrance to root growth. High soil bulk density in conjunction with low soil porosity can make root penetration difficult (Bengough and Mullins, 1990).

2.5.3 Determining fine root turnover

Although fine root production and mortality, collectively called turnover, has proven to be difficult to assess quantitatively, the benefits in understanding this essential process are substantial. With the advantage of increasing CO_2 sequestration and accessing soil nutrients, the rate at which fine roots change in their dynamic environment is key to understanding whole plant processes (Gill and Jackson, 2000; Majdi et al., 2005). Several methods have been developed to examine root turnover, each looking at specific aspects of the life cycle of fine roots. Production, mortality and longevity have all been assessed to ultimately determine the residence time of C in the soil. There is no current standard that is used to calibrate the methods (Majdi et al., 2005), and the methods utilized appear to produce somewhat conflicting results. Root longevity studies using isotope-based

procedures have yielded residence times as much as four years greater than those using the minirhizotron system (Strand et al., 2008). Helmisaari et al. (2007) attempted to quantify fine root turnover in Norway spruce and Scots pine stands by correlating it with site and stand characteristics. They found that fine root biomass ranged from 230 to 493 g m^{-2}, which is comparable to other fine root estimates of the same species. Although attempts have been made to synchronize the results from various methods, the dynamic nature of fine root turnover suggest that only the utilization of multiple different methods will allow for full understanding of the root system (Strand et al., 2008).

2.5.4 Fine root growth and soil temperature

Soil temperature is one of the primary environmental factors affecting the growth of a plant (Alvarez-Uria and Korner, 2007). Although the minimum and maximum temperatures for root growth vary among species, values reportedly range from 5 to 37 ºC (Kaspar and Bland, 1992). Deans (1979) identified a correlation between increasing soil temperature and fine root production followed by decreasing soil temperature and gradual decline in root production. Roots typically move along a soil profile according to a temperature gradient. As the growing season progresses, deeper layers increase in temperature and root growth becomes more closely related to changing soil temperature (Kaspar and Bland, 1992). However, Cote et al. (1998) found an inverse relationship between soil temperature and fine root growth. They suggested this trend to be an indirect consequence of the positive relationship between soil temperature and aboveground growth, which negatively affects belowground growth.

2.5.5 Allometric relationships

In a SRWC, an extensive root system is instrumental in determining the health of aboveground regeneration after coppicing. Understanding root biomass in relation to shoot biomass is central to the awareness of how carbon is allocated during different periods of growth. A relationship between above and belowground growth exists based on several environmental parameters and seasonality. Problems have arisen in trying to determine this relationship due to difficulties in turnover recognition, and the arbitrary assignment of the physical distinction between above and belowground biomass (Mokany et al., 2006). Variations observed in aboveground biomass have been attributed to differences in root: shoot partitioning (Martin and Stephens, 2006). Some ecosystems exhibit wide ranges in root: shoot ratios throughout a growing season. In an analysis of root to shoot relationships in terrestrial biomes, Mokany et al. (2006) found root: shoot ratios for shrub-land and grasslands to be much wider than forest and woodlands. Contrary to the

many studies that use biomass estimates to determine and predict carbon allocation, Litton et al. (2003) suggest that biomass is not a useful observation tool, as it only explains approximately 33% of the carbon flux in a plant.

2.6 Root Measurement Methods

Considering the simultaneous nature of fine root growth and senescence, different measurement techniques have been developed to separate these two processes (Rytter, 1999). The variety of *in situ* methods for quantifying fine-root turnover can be separated into two distinct categories: destructive and non-destructive (Vargas and Allen, 2008). Destructive methods constitute a majority of the measurement techniques and include soil coring and whole tree excavation (Achat et al., 2008; Rytter, 1999). These techniques provide only point in time root biomass estimates, are labor intensive, and cannot guarantee capture of all the fine roots in a system. Non-destructive methods include observation techniques such as the minirhizotron method which allow for multiple readings to be taken from the same location (Vargas and Allen, 2008). In a method comparison study, Rytter (1999) found that different methods of quantifying fine-root turnover produced various results.

2.6.1 Minirhizotron method

Since its introduction by Bates (1937), the use of the minirhizotron camera system in the study of roots has become a widely used observation technique to examine the dynamics of fine roots or supplement destructive observation methods (Hendrick and Pregitzer, 1996; Johnson et al., 2001; Majdi, 1996; Satomura et al., 2007). It involves the installation of transparent tubes into the ground 30 to 40^{o} from the horizontal and the insertion of a camera into the tubes to gather information about the surrounding root system (Bragg et al., 1983; Heerman and Juma, 1993). The efficiency of this method in the field has increased over time as a result of improving equipment and software (Vamerali et al., 1999). Of the many attractions to this method is its ability to observe the same roots sequentially over frequent time intervals is its primary advantage (Johnson et al., 2001; Samson and Sinclair, 1994). This allows for roots to be measured for production, mortality and turnover (Majdi, 1996).

Many studies have suggested that the installation and presence of the tube in the soil influences the growth of the surrounding roots. With minirhizotron imaging, it is important to consider how accurately the roots at the surface of the tube represent the bulk soil. Studies have demonstrated the tendency to underestimate the density of the roots in upper soil layers and

overestimate root density deeper in the soil profile (Bragg et al., 1983; Gregory, 1979; Volkmar, 1993). In a study conducted by Withington et al. (2003), European beech *(Fagus sylvatica)* roots and Scots Pine *(Pinus sylvestris)* roots were larger in length and diameter next to butyrate and acetate plastic tubes, respectively, than next to glass. This observation was largely considered to be a consequence of the chemical nature of the tube. Borosilicate glass tubes have a chemical structure that is closer in composition to the surrounding soil. Plastic tubes are not chemically similar to soil and may have a tendency to bleed some of the compounds into the surrounding environment. Regardless, plastic tubes are preferred over glass due to their flexibility and their close contact with the soil (Withington et al., 2003). Although polycarbonate tubes are less prone to scratching than other plastics, they are still susceptible to some degree of marking over long term use (Johnson et al., 2001).

A number of measures must be taken to ensure the environment surrounding the minirhizotron tube is representative of the bulk soil. The movement of the tube within the soil is detrimental to proper root turnover examination as movement may form an open space, an adventitious pathway for roots. Hence, it is vital that there is solid contact between the tube and soil (Johnson et al., 2001; Satomura et al., 2007). Roots tend to grow along a surface upon contact, overestimating rooting density at depth. Bragg et al. (1983) suggested that the minirhizotron tubes be inserted at a 30 to 45^{o} angle to the ground to avoid this. As well, it is vital to avoid trampling in the vicinity of the tube during installation (Phillips et al., 2000; Taylor et al., 1990). Post-tube installation, a root re-establishment period may be necessary to allow the root system to reach its undisturbed density surrounding the tube. The length of time required may last from six months to one year (Bernier and Robitaille, 2004; Hendrick and Pregitzer, 1996; Johnson et al, 2001).

Various software programs have been developed to analyze the minirhizotron images (Majdi, 1996). In the process of image analysis, measurements of the root length and diameter are taken and the roots are classified by colour and root order (Pritchard et al., 2008). Processing by tracing the images is an arduous task; however, this method still has many advantages over alternate methods of quantifying roots systems when a depth analysis is required (Cheng et al., 1991). Although the price of the minirhizotron system, including the camera equipment and software, can reach up to US$ 10,000 to 15,000, it is a relatively quick and reliable method for quantifying root turnover (Johnson et al., 2001).

2.6.2 Sequential soil coring method

The soil coring method is the most common method used for quantifying single point in time fine root biomass and NPP in the field (Vogt et al., 1998). It involves inserting a coring device (i.e. steel auger) of a known diameter down to a standard depth and extracting a calculable volume of soil. It is labor intensive, destructive and typically restricted to soils that are penetrable by the auger (Majdi, 1996; Makkonen and Helmisaari, 1999). Physical limitations of the soil environment may make sampling with an auger difficult or nearly impossible (Vogt et al., 1998). The roots samples are processed by the time-consuming task of washing and hand-sorting to gain quantitative values for root biomass (Schroth and Kolbe, 1994). Schroth and Kolbe (1994) proposed a quicker processing method involving homogenizing the sample and selecting a subsample as a representation of the whole. Using this method to reduce the processing time would allow for more samples to be taken per plot, aiding in mitigating the issue of site heterogeneity (Schroth and Kolbe, 1994).

Certain parameters of root systems such as growth rate and longevity are omitted with the use of this method (Johnson et al., 2001; Majdi, 1996), suggesting its use should be in conjunction with alternate root measurement methods such as the minirhizotron technique (Vogt et al., 1998). Due to the method's inability to simultaneously examine root production and mortality, an underestimation of root production typically occurs. Many authors also attribute this underestimation to sampling dates that do not coincide with seasonal maxima and minima, and unaccounted for losses due to sloughing and exudation (Neill, 1992). Singh et al. (1984) suggest that underground biomass sampling is extremely time sensitive accounting for peaks in production and mortality, and that overestimation or underestimation in biomass can result from variability in root biomass data. Optimal sampling dates for roots determined by the seasonal maxima and minima are, however, difficult to determine (Block, 2004).

2.6.3 Comparison of root measurement methods

The range of techniques currently in use to determine belowground biomass indicates that there are a wide variety of methods available. Depending on the specific objectives of the study, some methods are more suitable than others. Many studies recommend combining more than one method to thoroughly examine root systems. Problems arise, however, in the range of production and biomass estimates that are generated when the various methods are compared. Studies have identified the minirhizotron technique's tendency to underestimate rooting density in surface (0-30 cm) soil and overestimate rooting density at depths (30-60 cm) when compared to soil core data.

This discrepancy between the methods has been observed in maize (Samson and Sinclair, 1994), spring wheat (Bragg et al., 1983), winter wheat, and pearl millet (Gregory, 1979). This observation has been attributed primarily to site disturbance during installation (Jose et al., 2001) and preferential root growth along the surface of the tube (Gregory, 1979). Although some studies have suggested the discrepancy to be a result of inadequate equilibration time, others claim that this factor has little effect on the comparison of the two methods (Samson and Sinclair, 1994).

2.7 Conversion of Minirhizotron Data

There are a number of ways to analyze the data to produce meaningful values. Each type of interpretation is dependent on the root parameters that are being investigated. Typically, biomass estimates are done by a simple line-intersect method in which the roots are counted when they intersect a grid in the MR frame (Bragg et al., 1983; Gregory, 1979; Hendrick and Pregitzer, 1996). This procedure encourages a potential positive bias in root length diameter because a single root may cross lines in a grid several times, resulting in that root being counted more than once (Buckland et al., 1993). By calibrating the results against other methods such as soil coring, this error may be avoided.

2.7.1 Depth of field

Root length density (RLD) per unit volume of soil is a quantitative approach that is calculable using the depth of field method. This technique allows for root volume to be calculated from existing data by assuming that each window observed by the minirhizotron camera is three-dimensional. The third dimension, or "depth of field", is the area that extends out from the surface of the tube and is typically 2 to 3 mm wide (Johnson et al., 2001). This method has been utilized by various authors to cover more specific root parameters with minirhizotron data (Fitter et al., 1995; Jose et al., 2001; Steele et al., 1997). Due to the degree of non-uniform root distribution, or clustering, some authors have considered the RLD misrepresentative (Grabarnik et al., 1998; Lodgeson and Allmaras, 1991).

2.7.2 Plane-intersect method

The plane-intersect method is an adaptation of the line-intersect method and is an approach that utilizes root parameters obtained in image tracings to estimate root length density. This method was proposed by Merrill and Upchurch (1994), and is more suited to the three-dimensional minirhizotron environment than the simple line-intersect. The plane-intersect method determines root productivity by employing the most robust measurement parameters obtained using the

minirhizotron method, and aims to reduce the factor of interference by the minirhizotron tube itself (Bernier and Robitaille, 2004). To conceptualize, the soil in the tube is sliced into a number of infinite slices, and the root surface area in each slice is accounted for. Assuming that each slice is a proportional representation of the whole, the data can be extrapolated to determine fine root volume per unit soil volume.

2.8 Fine Root Carbon Capture and Storage

With knowledge of the impending global climate crisis, the potential for large amounts of carbon dioxide (CO_2) to be pulled from the atmosphere and placed in the long-term stores of forest systems has become a focal point in forestry research. Willow has the added contribution of being a carbon neutral alternative to fossil fuel sources. While, there is relatively little known about the potential of willow roots to store CO_2, it is understood that although harvesting of the aboveground portion of the system is paramount to the C sequestration potential for willow, fine root turnover also largely contributes to the strength of the crop as a CO_2 sink. Frequent coppicing aboveground encourages more rapid root turnover, therefore more C sequestration, than undisturbed woodland sites (Grogan and Matthews, 2002). Roots that are more resistant to the process of decomposition are an important part of the long-term contribution of fine roots to C sequestration.

By intensively managing these crops, farmers can increase belowground and aboveground storage, as well as levels of soil organic matter (Zan et al., 2001). Ruess et al. (1996) found that C allocation to fine roots ranged from 1.50 to 4.25 Mg ha^{-1}. According to a study done by Zan et al. (2001), crops may display varying relationships between belowground biomass and soil C storage. In their study, C storage was compared between switchgrass and willow and the soil under willow reported higher levels of soil C accumulation, despite similar belowground biomass. Grogan and Matthews (2002) found that the average rate of soil carbon sequestration for a willow plantation to a depth of 23 and 50 cm was 0.41 Mg C ha^{-1} yr^{-1} and 0.51 Mg C ha^{-1} yr^{-1}, respectively. A conceptual model was used by Grelle et al. (2007) to estimate C sink potential for a willow plantation in which fine root senescence was assumed to be equal to fine root growth. This study indicated that belowground C allocation was 2.97 Mg C ha^{-1} yr^{-1}, substantially higher than previous studies. A simulated model following current trends revealed that in 100 years, belowground biomass of willow could sequester as much as 20 Mg C ha^{-1} (Grogan and Matthews, 2002).

A review of pertinent literature has indicated that, although short rotation willow cropping systems provide substantial opportunities for various environmental and economic benefits for

landowners in Saskatchewan, there is a lack of necessary information on the belowground portion of the system. An assessment of the fine root dynamics of a willow plantation in Saskatchewan will allow researchers to make better management decisions regarding fine root growth and more accurate projections for fine willow root C sequestration in Saskatchewan.

2.9 References

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3.0 FINE ROOT BIOMASS, PRODUCTION AND TURNOVER OF SIX WILLOW CLONES IN SASKATCHEWAN

3.1 Introduction

Short rotation woody crops (*Salix* and *Populus* in particular) are currently used in parts of Europe and North America as a bioenergy source which can also reduce the impact of climate change on the environment. The ecological benefits associated with these crops include their positive effects on soil and water quality, and their lower overall levels of greenhouse gas emissions when compared to fossil fuels (Volk et al., 2006). Willow plantations have not been grown in Saskatchewan and examining the fine root dynamics of willow is needed for more accurate determination of the site conditions in which willow should be grown for maximum yields in Saskatchewan.

Not only do fine root systems in SRWC play an important role in nutrient accumulation, they can contribute as much as 50% of the annual net primary productivity (Rytter, 1999). However, obtaining accurate estimates of belowground biomass and productivity has provided measurement challenges, as monitoring the belowground portion of the plant system is a difficult task. As fine root growth and death occur simultaneously, frequent observations are required to document the rapid changes that occur in fine root systems. The minirhizotron method is a tool that was developed for this purpose because it provides temporal information on individual root systems.

The relatively new development of willow plantations as a bioenergy crop in Saskatchewan presents many site-specific questions regarding the success of these plantations. This experiment is the first attempt to utilize the minirhizotron method to understand willow fine root activity of six willow clones in Saskatchewan. The objective of this study was to determine willow fine root biomass, productivity and turnover using the minirhizotron method for six willow clones over two seasons of growth in Saskatoon, Saskatchewan. It was hypothesized that there would be a significant difference in fine root turnover, biomass and productivity among the six willow clones in Saskatoon using the minirhizotron method The null hypothesis states that there will be no significant effect of willow clone on fine root biomass, productivity or turnover.

3.2 Materials and Methods

3.2.1 Site description

One 0.6 ha (5972.4 m^2) willow plantation was established in the spring of 2007 at the University of Saskatchewan Horticulture Field Laboratory (52^o 7' 35.91" N, 106^o 36' 26.43" W) in

Saskatoon. This site is situated within the city limits on agricultural land in the Prairie Ecozone of Saskatchewan. It is a flat landscape that lies in the Saskatoon Plain region and is overlain by glacio-lacustrine material (Sutherland Association). It is part of the Dark Brown soil zone of Saskatchewan and is dominated by a heavy clay soil texture and classified as a Sutherland Orthic Vertisol. The bulk density of the surface soil in this area is 1.28 g cm^{-3}.

3.2.2 Experimental design and plantation establishment

This experiment was established as a randomized block design (n=4) where each block, or replicate, contains 30 willow clones arranged in 6.3 x 7.9 m plots. Each plot contains three double-rows with thirteen trees per row. Rooted cuttings were spaced 0.6 m within the row and between each row in the double row. The double-rows were spaced about 1.5 m apart and the outer rows in each plot acted as buffer zones, while the centre double row was used as the measurement plot. The cuttings were collected from one-year-old hybrid willow clones, originating from the willow program at the State University of New York College of Environmental Science and Forestry (SUNY-ESF) and were planted as unrooted cuttings. The cuttings were 25 cm long, with diameters ranging from 8 to 21 mm. They were kept at –4 ºC until planting, and were soaked immediately prior to planting. It is expected that this pre-planting treatment stimulates the rapid establishment of the root system (Pezeshki and Shields, 2006). Of the 30 clones, only 6 were chosen for observation in this study: SX61 (*Salix sachalinensis*), SX64 (*Salix miyabeana*), Fish Creek (*Salix purpurea*), Allegany (*Salix purpurea*), Sherburne (*Salix sachalinensis x miyabeana*) and Canastota (*Salix sachalinensis x miyabeana*).

The site was mechanically prepared by tilling using a tandem disc, Case IH 165 H.P. tractor on 17 May 2007 and was planted on 28 May 2007. The plantation was sprayed with the pre-emergent herbicide oxyflurofen (Goal®) (Rohm and Haas Co., Philadelphia, PA) at a rate of 2 L ha^{-1} immediately after planting. The post-emergent herbicide bromoxynil was applied on 17 July 2007 at a rate of 0.5 L ha^{-1}, as well as the herbicide glyphosate at a rate of 2 L ha^{-1}.

Planting depth of the cuttings was inconsistent due to the number of planters involved. Depth of the top of the cutting varied from 3 cm below the ground surface to 4 cm above the ground surface. After one growing season, the site was coppiced, or cut back to ground level in April 2008. Hence, MR data presented from May to September 2008 refers to one-year-old root systems under newly regenerating aboveground biomass. In May 2008, the herbicide Simazine 480 was applied to

the plantation at a rate of 7 L ha^{-1} using a BX2350 Kubota tractor and a 5.5 m boom sprayer. Weeds were removed by hand throughout the 2008 and 2009 growing seasons.

3.2.3 Minirhizotron tube installation

Minirhizotron tubes (n=18; 6 clones X 3 reps) were installed in July 2007 to examine the six willow species in replicates one, two and three. The tubes were placed within each of the measurement tree rows at randomly selected trees. The minirhizotron tubes are 5 cm in diameter and are composed of a transparent acetate-butyrate material. They were installed by using a steel coring tube with a reverse-taper bit with a diameter slightly larger (0.0254 mm) than the actual minirhizotron tube. The coring tube was inserted into the soil 38º from the horizontal using a PacePik model 2550 (Williams and James Engineers, Ltd., Gloucester, GB) hydraulic concrete breaker. This apparatus created a cylindrical into which the minirhizotron tube was inserted. The tubes reached a vertical depth of 30 cm, but varied due to difficulties upon insertion of the coring device into the heavy clay soil**.** The tubes were firmly secured in place with the installation of two metal rods into the soil immediately adjacent to the minirhizotron tubes. The rods were tightly fastened to the tubes using plastic cinch-ties in order to minimize tube movement in the soil. Subsequently, the exposed upper portion of the tube (approximately 20 cm in length) was painted black to eliminate light entry, and then painted white to minimize heating by the sun. To ensure minimal moisture leakage in the tube, rubber stoppers were used to cap the ends, and then covered with aluminum cans and plastic bags.

3.2.4 Minirhizotron imaging and analysis

Minirhizotron images were collected monthly from May 2008 to October 2008, and May 2009 to October 2009. The camera system is comprised of a specialized digital camera attached to an indexed handle (Bartz Technology Co., Santa Barbara, CA, USA). This technique involved lowering the camera into the minirhizotron tubes. To ensure the images were consistently taken at the top of the tube, the camera was secured in place by locking it in a standard position at the top of the tube. Images were captured at 1.2 cm increments along the upper surface of the tube, guided by indents on the indexed handle. The images were saved using Bartz technology's I-CAP image software onto a Sony field computer where they were stored until laboratory analysis on an alternate computer.

Throughout the 2008 and 2009 growing seasons, approximately 17,000 images were taken: the equivalent of approximately 96 images per tube throughout the course of 10 image-capturing sessions. Examples of the collected images can be found in Appendix A. Analysis of the images

involved tracing the roots that appeared in each image using RooTracker software (Version 2.0, Duke University, NC, USA). Most roots were classified as fine roots (diameter of ≤ 2 mm) and the lengths and diameters of each root were recorded. Each set of images was traced in sequential order to chronologically determine the rate of root growth and die back. In order to examine root dynamics in 10 cm vertical increments, a sine equation (10 cm / sine 38°) was used to calculate the length of tube that was equivalent to 10 cm vertical depth. This value was then divided by the distance between each image on the tube (1.2 cm) to determine the number of images that are contained within a 10 cm vertical depth. Based on this formula, 13 sequential images describe fine root activity information for a 10 cm vertical section.

Comparison of root growth among each clone required a common tube depth among the tubes. To accomplish this, tube information was truncated for each tube to match the shallowest tube depth. The minimum image was subtracted from the maximum image for each tube. The smallest number of images was used as the new standard for each tube. Information was deleted for images below this depth.

3.2.5 Conversion to bulk soil estimates

Transformation of minirhizotron productivity data to reflect root productivity in the bulk soil is carried out using the plane-intersect method, developed by Bernier and Robitaille (2004), described in Chapter 2. The method utilizes the two most reliable root parameters determined by the minirhizotron method: date of appearance and root diameter. The plane-intersect method depicts the roots as a series of ellipses, whose long axis is dependent on the diameter of the root and the angle of incidence. An equation is utilized to determine the expected area of the ellipses. Assumptions carried over from the line intersect method developed by Van Wagner (1968) yield the sum of the expected elliptical cross sections (A_e) of roots crossing the plane delineated by the minirhizotron tube surface, defined in the following equation:

$$\Sigma A_e = \frac{\pi^2 \Sigma r^2}{\sqrt{2}} \qquad (3.1)$$

Applying this equation to all new fine roots, fine root productivity (P_{fr}) (g m^{-2}) is calculated as:

$$P_{fr} = 2(10^6)\, \rho_{fr}\, (1 - F_c)\, \Sigma A_e\, \frac{\sin \alpha \cos \gamma}{W}, \qquad (3.2)$$

where ρ_{fr} is the specific mass of willow roots (g mm^{-3}). F_c is the fraction of coarse stone fragments in the soil (0, as determined by soil profile examination), ΣA_e is the sum of the expected elliptical cross sections of all new fine roots that have appeared since the last imaging date as calculated in equation

3.1, α is the angle of the tube relative to the ground (38°), γ is the ground angle relative to the horizontal (0°), and W is the width of the minirhizotron frame (14.2 mm). The factor of 2 X 10^6 is used to convert the values from three dimensions to two for the purpose of planar interpretation. Statistical Analysis Software (SAS) (Version 9.1, SAS Institute Inc, Cary, NC, USA) was utilized to apply the plane-intersect method. Net primary productivity (NPP) was calculated for each willow clone used in the experiment by summing the productivity values from May 2008 to May 2009.

The root and tube information is divided into two input files upon implementation of the plane intersect method. Productivity is computed using equations 3.1 and 3.2 through a series of steps in the form of a SAS script (Appendix B). The first input file includes root information gathered during the tracing process such as tube number, frame number, sampling date, and root diameter. The second input file includes information on the physical soil environment and root characteristics such as tube number, angle of the tube relative to the ground, ground slope, stone fraction of the soil, and average specific root mass. The output files contain information on fine root biomass (g m^{-2}) and fine root productivity (g m^{-2}). The values were converted to Mg ha^{-1} for the purpose of this study.

3.2.6 Average specific root mass calculation

An assessment of specific root mass was required for the second input file for SAS and was calculated for each willow clone. A section of willow root for each clone, obtained during whole tree excavations (methodology described in section 4.2.4 of this thesis), was weighed then submerged in a graduated cylinder containing a known quantity of water. The weight (g) was divided by the volume of water displaced by the root (mL). This provided the specific root mass for each willow clone in g cm^{-3}.

3.2.7 Fine root longevity and turnover calculation

Fine root turnover is a key component in the assessment of ecosystem carbon cycling and is defined as the proportion of standing root biomass replaced annually (Majdi et al., 2005; Pregitzer et al., 2000). Rates of fine root turnover are dependent upon edaphic factors such as soil temperature and nutrient status, and genotypic determination. Several equations have been presented to calculate this value using known root parameters of production and mortality. They are largely based on fine root length and arbitrarily defined root diameter classes. (Bernier and Robitaille, 2004; Guo et al., 2008; Pregitzer et al., 2000). Guo et al. (2008) discussed the potential limitations of the diameter class approach, particularly in the area of root mortality designation, and suggested the adoption of a

root-order-based approach to calculating fine root turnover. In minirhizotron studies, fine root turnover is defined as the inverse of median longevity. Due to the difficulty in determining the mortality among fine roots, (Bernier and Robitaille, 2004; Hendrick and Pregitzer, 1996a) diameter and date of appearance were used for the productivity estimate. Longevity is defined as the length of time it takes for a group of fine roots to turnover once. For the purpose of this study, fine root turnover was calculated by determining the net annual productivity value and then dividing by the maximum fine root biomass value (Gill and Jackson, 2000). Longevity was, in turn, calculated as the inverse of fine root turnover as follows:

$$L_{fr} = 1/T_{fr} \quad (3.3)$$

where L_{fr} is fine root longevity (yr) and T_{fr} is fine root turnover (yr^{-1}).

3.2.8 Other measurements

Beginning 24 July 2008 and continuing until 22 September 2009, measurements of soil temperature were recorded at each tube using HOBO® H8 Temperature data loggers (Onset Computer Corporation, MA) (n = 18). The probes were located approximately 60 cm from the tubes and were placed at 10-and 30-cm depths. Daily average temperature was calculated to determine the number of growing-degree days (GDD) using a base temperature of 5ºC (Kopp et al., 2001). Above ground growth was measured in order to better understand the relationship in the rate of growth between the shoots and the roots of willow trees. This information is key to understanding photosynthetic investment of aboveground and belowground portions of the plant (Titlyanova et al., 1999), and the plants growth response to the environment, although the mechanisms at work determining root: shoot ratio are variable. Root collar diameter (RCD) was used as the aboveground growth parameter. One tree was selected directly to the right of each tube and upon each image capturing session, the diameter of a single randomly selected marked stem was recorded (mm).

3.2.9 Statistical approach and analysis

The minirhizotron study was analyzed as a repeated measures nested design. As with most minirhizotron studies, repeated measurement was considered a factor in the creation of the statistical model in order to compare the response curve over time (Littell et al., 1998). Each data collection session was considered a repeated measurement for each experimental unit. In each analysis, the clones were treated as independent fixed variables. SAS (Version 8.0, SAS Institute Inc. Cary, NC, USA) was used to implement the plane-intersect method (see Appendix B) to obtain biomass and

productivity values from the raw minirhizotron data. The output from the plane-intersect method displayed non-normal distribution. To correct this, all data were natural log transformed but were presented as untransformed data. The dataset was subject to univariate and multivariate analyses of contrast variables using a linear contrast in SAS, as an ANOVA might have provided invalid results due to the covariance structure of the data. Statistical analysis was done using a 95% confidence interval ($P < 0.05$). However, turnover and longevity significance testing was completed with an ANOVA in the statistical program R (Hornik, 2008).

3.3 Results

3.3.1 Change in total root number

In May 2008 there were a total of 144 roots for all the clones observed using the minirhizotron method to a depth of 40 cm (Figure 3.1). By September 2008, the total number of roots increased significantly to 1217, and then decreased during the winter to 668 in May 2009. The number of roots increased again to 7374 roots by September 2009. The clones did not significantly differ in terms of total number of roots within a sample period. Overall, however, there were notable differences seen among the sample dates. Root numbers significantly increased from month to month throughout both growing seasons with the exception of the August to September 2008 sampling time.

3.3.2. Root biomass

Fine root biomass gradually increased for most of the clones between May 2008 and September 2009 (Figure 3.2). Monthly fine root biomass averaged for all clones ranged from 0.78 ± 0.78 Mg ha^{-1} May 2008 to 25.75 ± 9.11 Mg ha^{-1} in September 2009 (Figure 3.2). Fine root biomass values increased at a slower rate through the 2008 growing season compared to the 2009 growing season. The most pronounced period of biomass increase was between June and July 2009. All clones increased in biomass each month in 2009 with the exception of SX64, which decreased slightly between July 2009 and August 2009. Significant differences in root biomass when all clones were averaged were seen between each month within the two growing seasons, but not between September 2008 and May 2009. Root biomass did not significantly differ between the clones throughout the 2008 and 2009 growing seasons.

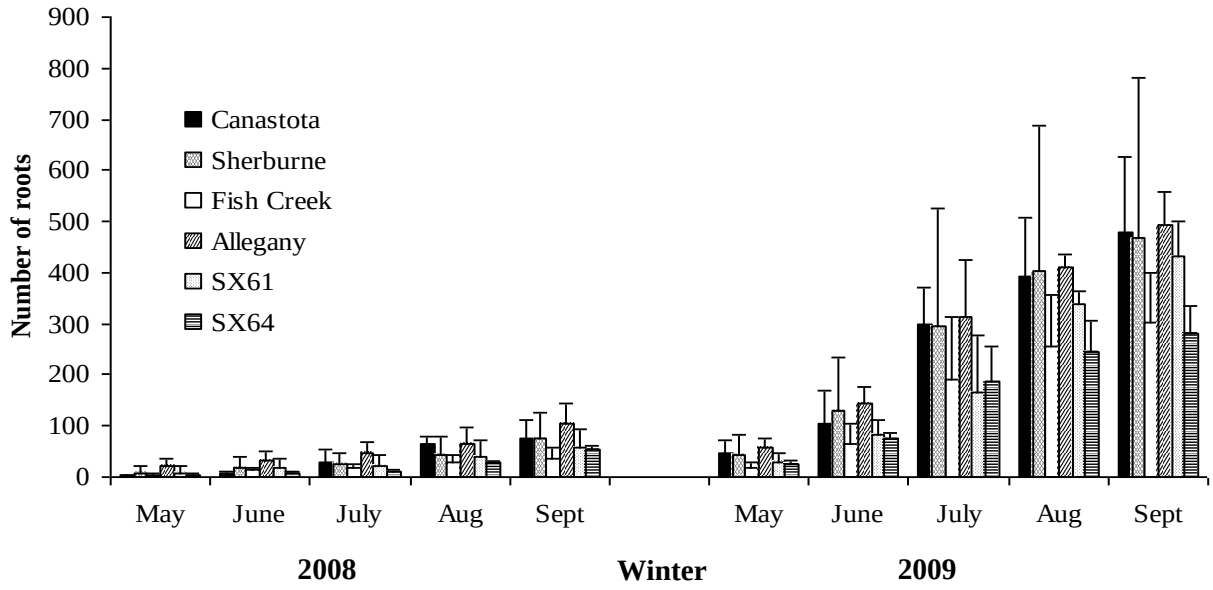

Figure 3.1 Average total (n = 3) number of roots for each clone from May to September for the 2008 and 2009 growing seasons. Although there was no significant clonal effect, overall significant differences were seen between each sample period with the exception of the time between August 2009 and September 2009 ($P < 0.05$). Analysis was performed on log transformed data. Vertical bars indicate one standard deviation.

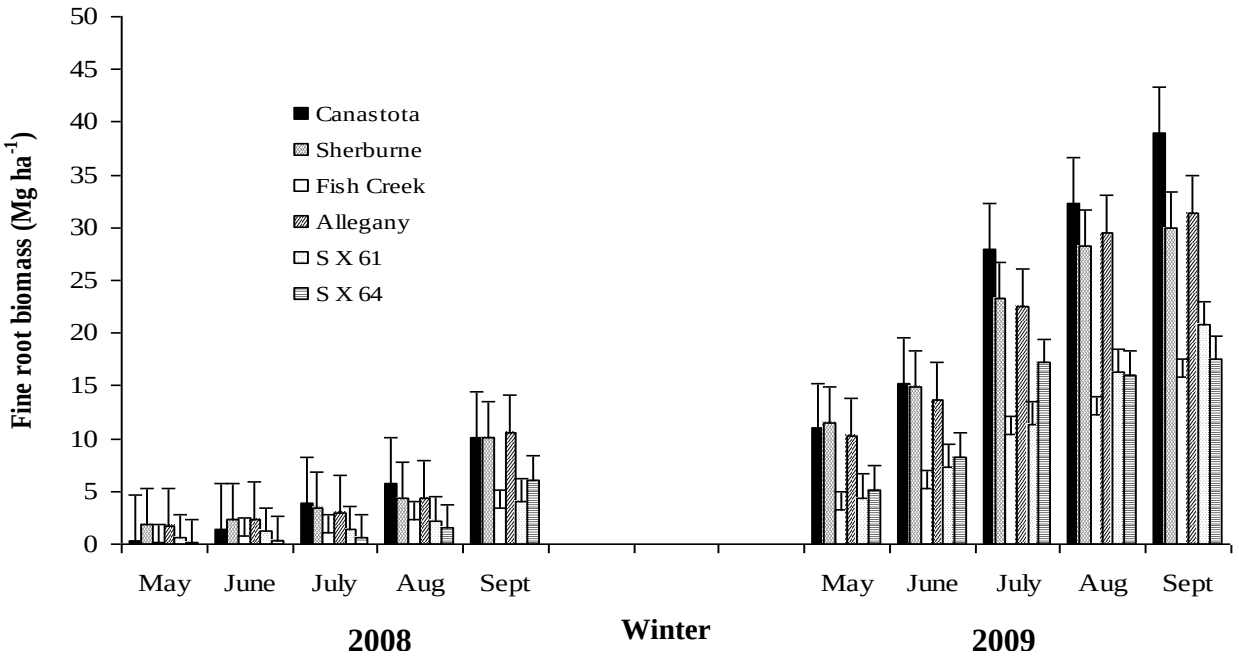

Figure 3.2 Minirhizotron estimates (n = 3) of fine root biomass for each clone over 2008 and 2009 growing seasons. Although there was no significant clonal effect, overall significant differences were seen between each sample period with the exception of the time between September 2008 and May 2009 ($P < 0.05$). Analysis was performed on log transformed data. Vertical bars indicate one standard deviation.

3.3.3 Fine root NPP

Throughout the two growing seasons, monthly NPP did not show any differences between the clones (Figure 3.3). However, when averaging all clones together, monthly fine root NPP reached its peak for 2008 in September (3.39 ± 1.76 Mg ha^{-1}) which was a significant increase from August. A significant drop in fine root NPP was observed between September 2008 and May 2009 but monthly NPP significantly increased each month from May 2009 to July 2009, when it reached its peak of 8.00 ± 3.12 Mg ha^{-1} for 2009. A significant reduction in fine root NPP was observed from July 2009 to August 2009.

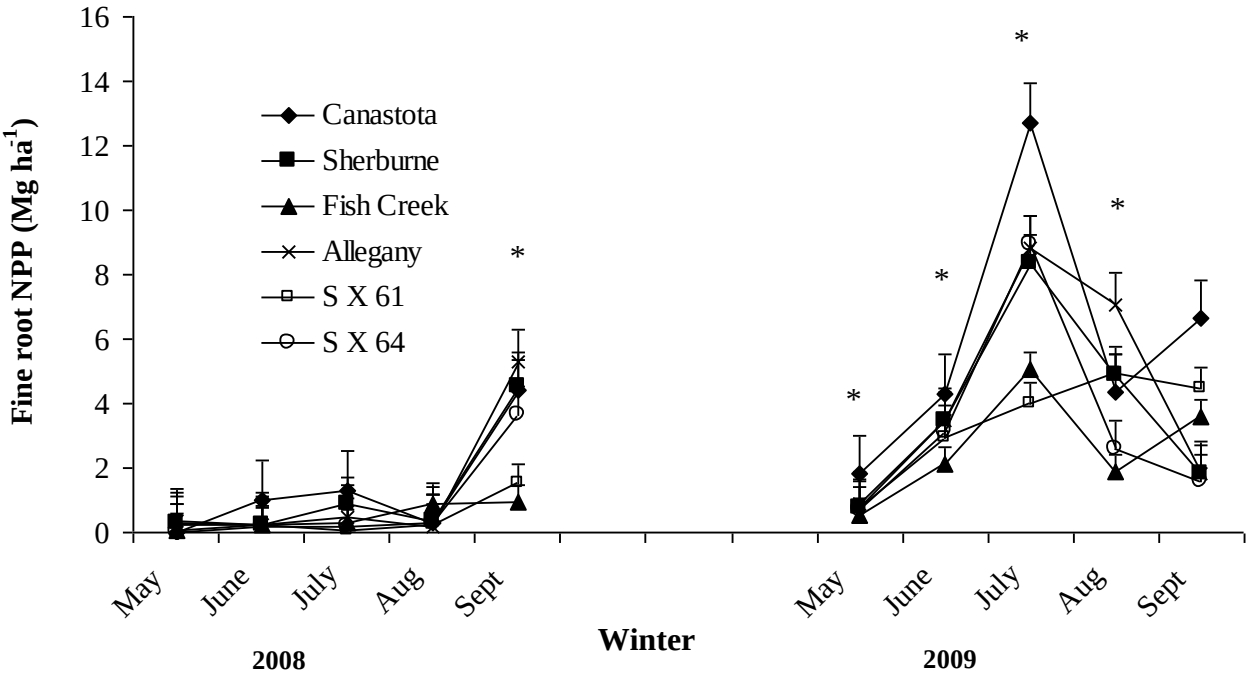

Figure 3.3 Minirhizotron estimates (n = 3) of monthly fine root net primary productivity of each clone over 2008 and 2009 growing seasons. * indicates a significant change between that month and the previous month that was sampled when all clones were averaged together ($P < 0.05$). Vertical bars indicate one standard deviation.

3.3.4 Fine root biomass and soil temperature

Due to technical problems, soil temperature data are only available for the growing season of 2009. Averaging GDD for all the clones, June had the highest monthly GDD (439) at 10 cm whereas July had the highest monthly GDD (361) at the 30 cm depth (Table 3.1). There was a similar increase in GDD for both the 10 and 30 cm depths from May to June. A gradual decline in monthly GDD commences in June at the 10 cm depth, and July at the 30 cm depth. Figure 3.4 shows a positive

relationship between soil GDD and mean fine root biomass. See Appendix C for a graph of monthly soil GDD.

Table 3.1 Soil growing degree days (n = 3) for each clone at each month from May to September 2009 collected using HOBO® H8 Temperature data loggers.

Clone	Growing degree days				
	May	June	July	August	September
	----------10 cm----------				
Canastota	292	453	428	388	287
Sherburne	273	443	433	391	177
Fish Creek	271	450	452	426	308
Allegany	284	420	410	399	299
SX61	251	445	454	403	285
SX64	279	424	417	387	282
Average	275	439	432	399	293
	----------30 cm----------				
Canastota	140	313	362	341	250
Sherburne	135	313	361	341	253
Fish Creek	126	300	354	342	253
Allegany	132	296	348	346	259
SX61	128	304	368	347	254
SX64	157	319	377	363	263
Average	136	307	361	347	255

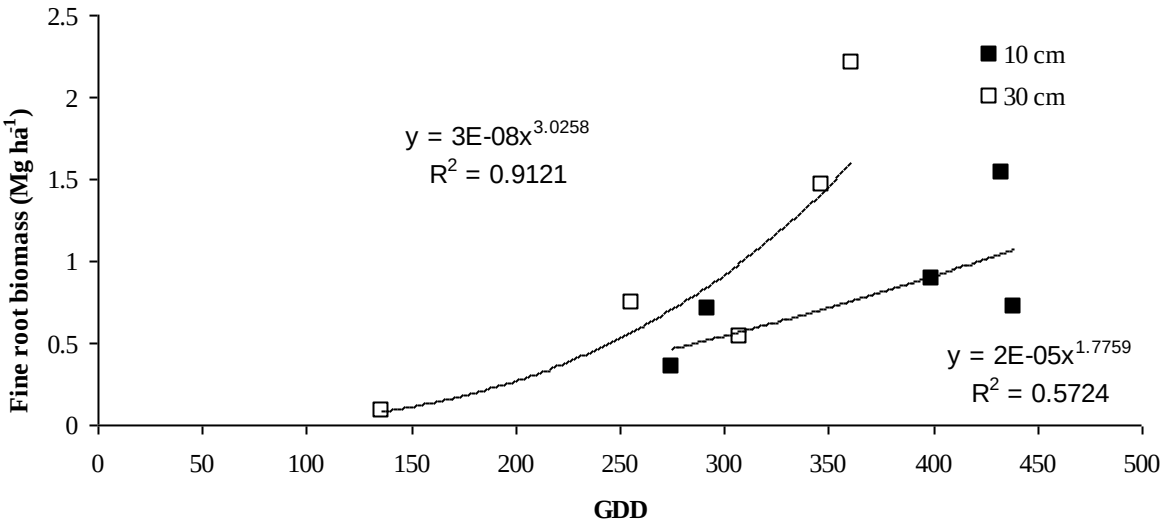

Figure 3.4 Relationship between monthly soil growing degree day (n = 3) and monthly fine root biomass from May to September 2009 at 10 and 30 cm depths. Regression line fit using a power function.

3.3.5 Depth profile distribution

There was no clear relationship between fine root biomass and soil depth for any of the clones (Table 3.2). The mean value of the clones at each depth showed there to be greater fine root biomass at the 10 to 20 cm depth than at the other depths. The mean value of the clones at this depth for 2008 and 2009 was 2.65 and 8.07 Mg ha^{-1}, respectively. There was a significant increase in the mass of roots at all depths from 2008 to 2009 (Table 3.2). A linear contrast in SAS showed no significant effect of either clone or depth on fine root growth.

3.3.6 Fine root biomass and root collar diameter

There was a positive relationship between RCD and belowground biomass for each clone where increasing stem diameter corresponded to an increase in root biomass (Table 3.3). Refer to Appendix D for a graph of RCD and belowground biomass relationship growth.

Table 3.2 Depth distribution (n = 3, SD in parentheses) of fine root biomass (Mg ha^{-1}) for each clone in 2008 and 2009. No significant relationship was found between the clones and the depths and a clone-depth interaction using a linear contrast in SAS ($P < 0.05$).

	Root Biomass						
Depth	Canastota	Sherburne	Fish Creek	Allegany	SX61	SX64	Average
	----------Mg ha^{-1}----------						
(cm)				2008			
0-10	0.80 (0.30)	0.95 (0.69)	1.46 (1.71)	2.21 (0.84)	2.61 (1.42)	1.78 (0.97)	1.64 b †
10-20	7.31 (9.79)	4.22 (6.51)	0.83 (0.64)	2.09 (1.31)	0.85 (1.17)	0.60 (0.40)	2.65 b
20-30	0.07 (0.61)	4.39 (6.71)	0.29 (0.18)	1.29 (0.78)	0.39 (0.08)	1.66 (1.05)	1.61 b
30-40	1.93 (1.57)	0.49 (0.57)	0.77 (0.12)	5.03 (7.41)	0.37 (0.49)	1.88 (2.72)	1.75 b
				2009			
0-10	6.09 (5.07)	6.09 (4.25)	3.09 (1.41)	4.48 (1.91)	7.97 (4.33)	5.79 (2.84)	5.59 a
10-20	21.27 (9.26)	8.94 (11.00)	3.70 (2.26)	5.85 (1.45)	5.16 (3.35)	3.52 (3.33)	8.07 a
20-30	4.57 (2.10)	12.00 (18.40)	5.20 (0.77)	8.01 (9.03)	3.81 (2.64)	4.78 (0.97)	6.40 a
30-40	7.02 (5.97)	3.03 (2.93)	3.85 (1.34)	13.1 (13.1)	3.84 (3.12)	3.36 (2.14)	5.73 a

† Means followed by the same letter in a column are not significantly different ($P < 0.05$) using a linear contrast in SAS.

Table 3.3 Regression relationship (n = 3) using a power function between root collar diameter (mm) on the X axis (30 cm from base) and belowground biomass (Mg ha^{-1}) on the Y axis.

Clone	Regression equation	R^2
Canastota	$y = 0.085x^{2.0771}$	0.900
Sherburne	$y = 0.179x^{1.9041}$	0.934
Fish Creek	$y = 0.003x^{3.1081}$	0.963
Allegany	$y = 0.076x^{2.2058}$	0.931
SX61	$y = 0.007x^{2.9699}$	0.910
SX64	$y = 0.006x^{3.0529}$	0.814

3.3.7 Fine root turnover and longevity

Root turnover for all the clones ranged from 0.91 to 1.10 yr^{-1} and mean fine root turnover was 0.95 ± 0.05 yr^{-1} (Table 3.4). Root longevity ranged from 0.91 to 1.10 years and mean fine root longevity was 1.06 ± 0.06 years. A general linear model ANOVA in R showed there was no significant difference in either turnover or longevity between the clones.

Table 3.4 Turnover and longevity rates (n = 3, SD in parentheses) for each clone calculated from September 2007 to September 2009. A general linear model in R showed no significant difference in turnover rates between the clones in either turnover or longevity ($P < 0.05$).

Clone	Mean root turnover	Mean root longevity
	----------yr^{-1}----------	----------yr----------
Canastota	0.94 (0.03)	1.06 (1.06)
Sherburne	0.91 (0.07)	1.10 (0.03)
Fish Creek	0.98 (0.08)	1.02 (0.08)
Allegany	0.91 (0.02)	1.10 (0.02)
S X 61	0.93 (0.04)	1.07 (0.04)
S X 64	1.10 (0.11)	0.91 (0.10)

3.4 Discussion

3.4.1 Biomass and NPP

Although fine root biomass values determined by the minirhizotron method are variable in the literature, the values presented in this study were overall greater than fine root biomass values

reported for other SRWC. Volk et al. (2001) estimated fine root biomass in a dense willow stand of a similar age to be 3.72 Mg ha^{-1}. In a poplar plantation in Wisconsin, USA, Coleman et al. (2000) found fine root biomass to be 3.56 Mg ha^{-1}. The estimates in this study agreed with the values in the other studies between the period of May 2008 and August 2008. However, between the period of September 2008 and September 2009, the estimates in this study were two to six times greater than those found in the literature, reaching up to 25.75 Mg ha^{-1}. The soil core fine root biomass estimates discussed in Chapter 4 of this thesis were approximately 95% lower than the corresponding minirhizotron estimates. A similar comparison done by Rytter (1999) found soil core NPP to be 65 to 75% lower than minirhizotron estimates in basket willow.

Similar to fine root biomass, monthly NPP values for this study in 2008 were more similar to those in the literature than the monthly NPP values in 2009. Averaging the clones, monthly fine root NPP in 2008 ranged from 0.15 to 3.39 Mg ha^{-1}, which agrees with fine root monthly NPP determined by Gunderson et al. (2008) in a hybrid poplar plantation. In 2009, monthly NPP was greater, ranging from 0.91 to 8.00 Mg ha^{-1}. Annual fine root NPP from May 2008 to May 2009 was 5.71 Mg ha^{-1} yr^{-1}. Rytter (2001) determined annual fine root NPP in basket willows grown in a clay soil to be 6.31 Mg ha^{-1} yr^{-1}. Rytter (2001) also found annual fine root NPP values ranging from 2.7 to 6.5 Mg ha^{-1} yr^{-1}, which are similar to the annual NPP value found in our study. In contrast, annual NPP determined using alternative methods such as sequential soil cores and ingrowth cores have provided smaller values than those found in our study, such as 2.5 Mg ha^{-1} yr^{-1} (Ostonen et al., 2005), and 1.3 Mg ha^{-1} yr^{-1} (Steele et al., 1997) possibly due to the tendency of these methods to exclude the simultaneous nature of fine root growth and mortality.

The higher fine root biomass values found in this study may be explained by limitations of the minirhizotron method that have been discussed in the literature. Upon installation of the minirhizotron tubes, any roots that were in the pathway of the coring device were severed, possibly leading to root pruning and root proliferation (Joslin and Wolfe, 1999). It is possible this could have slightly contributed to the increased root biomass observed in this plantation; however, the tubes were installed within two months of planting the willows and it is unlikely that this would explain the overestimation of fine root biomass. Another factor to consider is the effect of the chemical nature of the minirhizotron tube on the surrounding soil. For ease in working with and transporting the tube, plastics (acrylic and butyrate) are preferred over glass due to the malleability of the former. However, plastic materials tend to have a greater chemical effect on the environment immediately adjacent to the tube, to which root growth would respond. Withington et al. (2003) found that the

accumulation of phenolic compounds surrounding butyrate tubes contributed to the higher rates of pigmentation seen in roots than those surrounding glass tubes. This study also found that standing crop root biomass observed through butyrate tubes was unusually high, but suggested this was an artifact of the tube. In a study done by Taylor and Bohm (1976) comparing glass and acrylic tubes, soil adhesion was found to be greater against glass tubes than acrylic tubes. This resulted in greater rooting density along acrylic tubes, which was attributed to poor soil/tube contact (Taylor and Bohm, 1976).

The physical nature of the environment at the soil-tube interface where the images are collected may drastically misrepresent natural conditions in the bulk soil (Taylor et al., 1990). This may depend on the installation process, soil environment and mobility or immobility of the tubes. In order to ensure representative conditions of the bulk soil, it is imperative that there is no gap between the soil-tube interface because gaps can lead to preferential rooting paths for fine roots (Gijsman et al., 1991; Hendrick and Pregitzer, 1996b). This potential gap at the tube-soil interface has also been considered to contribute to errors in the root tracing process as it can lead to reduced visibility of the fine roots in the images (Gijsman et al., 1991). The gaps may also be a focal point for bunches of roots to form at or track along the surface of tube (Upchurch and Ritchie, 1983). As these tubes were installed in a Vertisol, a soil type susceptible to seasonal shrinking and swelling, it is highly likely that tube movement occurred in the soil, thus leading to large gaps at the soil tube interface. An Australian study examining wheat and canola root growth following the removal of a lucerne crop found a much deeper and bulky root system in a Vertisol than in a Kandosol, although the authors did not mention that gaps were present next to tubes from tube movement as a factor (Moroni et al. 2006). Kandosols are reddish, strongly weathered soils probably equivalent to Ultisols of Soil Taxonomy. It was noticed in our study that bending of three tubes had occurred due to soil movement of tubes because there was resistance when inserting the camera into the tube. In one case the bending was extreme, which was indicated by the force needed to insert the camera to the base of this tube compared to the other tubes.

The appearance of moisture droplets in the images indicates the presence of gaps between the soil and the tube (Smit et al., 2000). These droplets were present in some of the images in this study leading us to believe that the overestimation of fine root biomass is a direct result of preferential rooting pathways. Additional sunlight entering through the gaps between the tube and soil and the cracks that are present in a dry Vertisol may substantially alter the soil environment around the tube, optimizing growth conditions and increasing root growth (Smit et al., 2000).

3.4.2 Soil temperature and depth profile distribution

The interpretation of the relationship between fine root growth at depth and soil temperature is confounded by the relationship between soil temperature and drought conditions (Pregitzer et al., 2000). Typically, an increase in soil temperature leads to increased root respiration and growth (Burton et al., 1998; Steele et al., 1997; Zogg et al., 1996). The soil warms to greater depths in the profile as the season progresses, encouraging deeper root growth, if there are no additional limiting factors. Kaspar and Bland (1992) found that the elongation of the roots at depth was a function of the soil temperature at that depth (Kaspar and Bland, 1992). This study found that there was a sharp increase in soil temperature between May and June 2009, and this increase was at a similar rate for both 10 and 30 cm depths.

A depth profile analysis of fine root biomass in this experiment showed there to be no significant partition in root biomass to a depth of 40 cm, similar to other studies (Hendrick and Pregitzer, 1996b). Some studies indicated an increase, then a decrease in root density with depth (Liedgens and Richner, 2001; Nicoullaud et al., 1994), and this pattern appeared to occur in the clones Canastota, Sherburne and Fish Creek in this study. This diverges from the current understanding that the majority of roots grow close to the surface and substantially decrease with soil depth (Hendrick and Pregitzer, 1996b; Kummerow et el., 1990). In a study of willow fine root distribution, Rytter and Hansson (1996) found a majority of the roots located in the upper 10 cm of the soil profile throughout the 1988 and 1989 growing seasons (Rytter and Hansson, 1996). Contrasting patterns in root biomass allocation at different depths do emerge, however, as root diameter has been found to increase with soil depth (Peek et al., 2006).

3.4.3 Belowground and aboveground growth

In belowground and aboveground growth comparisons, there is generally a lag period for root expansion following shoot elongation (Iivonen et al., 2001), which is related to the increased water and nutrient requirements of the aboveground portion of the plant at the beginning of the growing season during canopy expansion (Majdi et al., 2005). The allometric relationship between aboveground and belowground biomass has also been suggested to be a function of age, as hybrid poplar trees were found to distribute less biomass belowground and more to the aboveground portion as the trees increased in age (Wullschleger et al., 2005). The fine root biomass in our study appears to hold a close positive relationship with stem diameter. As allometric studies are often useful for determining the unseen portion of the plant in areas where direct observation is limited (Gargaglione

et al, 2010), this relationship between stem diameter and root biomass is meaningful. It is also thought that water and nutrient restrictions in the soil result in greater partitioning of C to the belowground biomass than the aboveground biomass (Nixon et al., 2001). This, however, was not the case when comparing total belowground to aboveground biomass, as the root to shoot ratio indicated that there was much more aboveground biomass than belowground.

3.4.4 Fine root turnover and longevity

Fine root turnover in this study ranged from 0.91 to 1.1 yr^{-1}, which is slower than findings from another willow root turnover study in which Rytter and Rytter (1998) determined turnover values to range from 4.9 to 5.8 yr^{-1} in a willow plantation in Sweden. These higher values were suggested by the authors to be due to the reduction of water and nutrient restrictions in the fast growing plantation (Rytter and Rytter, 1998). The turnover values in this study do, however, fall within the range of turnover values found for a northern hardwood forest of 0.7 to 2.0 yr^{-1} (Burke and Raynal, 1994) and for a mixed stand of 0.45 to 2.19 yr^{-1} (Nadelhoffer et al., 1985). The minirhizotron method has been found to overestimate fine root longevity and underestimate fine root turnover when turnover rates in a sampled population are heterogeneous (Guo et al., 2008). This is due to unaccounted birth and death of smaller roots that may occur between sampling dates, which could be a factor to consider when determining fine root turnover and longevity in this study.

3.5 Conclusion

This study showed that there may be some limitations to using the minirhizotron system for sampling fine roots in Vertisolic soils. Similarities were found between fine root biomass in 2008 in this study and other literature studies; however, in 2009, biomass appeared to far exceed any fine root biomass values found in the literature. It is likely that fine root biomass was overestimated due to issues with the tubes bending in Vertisolic soils, creating large gaps between the soil and tube which resulted in preferential root growth. This factor needs to be considered by future researchers using the minirhizotron method in Vertisols so that accurate estimates of fine root dynamics are gathered.

The overestimation of fine root biomass in this study produces barriers to identifying relationships between willow fine root dynamics in Saskatchewan and environmental controls, although fine root biomass did appear to be responsive to soil temperature. As moisture and temperature are often related, moisture may be another key factor to consider, and further research

examining the effects of soil moisture on willow fine root dynamics may provide valuable insights into willow fine roots in Saskatchewan.

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4.0 ESTIMATION OF FINE ROOT BIOMASS AND CARBON SEQUESTRATION OF SIX WILLOW CLONES IN SASKATCHEWAN

4.1 Introduction

The current environmental, economic and social pressures of finding alternative energy sources to fossil fuels is directing research towards seeking a better understanding of the individual components of a bioenergy system. The idea of bioenergy has been around for centuries, since early human civilization burned wood to heat their homes. Conversion technologies have become more advanced since then as the scope of the end product has broadened and the production of ethanol and other liquid fuels are currently being developed (Blanco-Canqui, 2010; McKendry, 2002). Along with the economic benefit associated with rapidly growing short rotation woody crops, they have the potential to sequester C for long term storage in their extensive root systems (Sanchez et al., 2007; Smith, 1995), and ability to remediate contaminated soil (Rockwood et al., 2004). Examining each portion of the whole plant system separately, such as shoots and roots, has led to specialization in specific areas of the bioenergy system and advancement of the methods used to carry out their individual examination. Maximum production and efficiency of the plantation is ensured by compiling information from all carefully examined portions of the plant system. As the root system, among other benefits, is integral to the vitality of the aboveground portion of the system, specific information on root systems may be considered fundamental for bioenergy cropping systems.

Short rotation willow crops can play a role in the efforts to reduce atmospheric carbon. Specifically, fine roots are often a focal point of these studies as they represent approximately 60% of total willow root C (Grigal and Berguson, 1997; Zan et al., 2001). With the relatively new establishment of willow plantations in Saskatchewan, obtaining belowground C sequestration values is important in the development of the C budget model and assessing the life cycle of willow in Saskatchewan. This life cycle assessment is important to the understanding of the environmental and socio-economic benefits of willow crop establishment in Saskatchewan. The primary objective of this study was to determine fine root biomass using the sequential soil coring method, and determine the amount of C being sequestered belowground for willow crops in Saskatchewan. The secondary objective of this study was to determine the coarse root structure of willow root systems using the whole tree excavation method. It was hypothesized that there would be a significant difference in fine root biomass and C sequestration among the six willow clones and among the four sites using the sequential soil coring method. The null hypothesis states that there would be no significant effect of willow clone or site on willow root biomass or root C sequestration.

4.2 Materials and Methods

4.2.1 Site description

Four sites in Saskatchewan were selected for this portion of the willow root biomass study: Saskatoon, Estevan, Prince Albert and Birch Hills. These sites were selected to represent a gradient of climate and soil conditions across the province. Unrooted willow cuttings of 30 clones were obtained from the willow program at the State University of New York College of Environmental Science and Forestry (SUNY-ESF). The plantation in Saskatoon contains all 30 clones while the other three plantations contain only six clones: Canastota (*Salix sachalinensis x miyabeana*), Sherburne (*Salix sachalinensis x miyabeana*), Fish Creek (*Salix purpurea*), Allegany (*Salix purpurea*), SX61 (*Salix sachalinensis*), and SX64 (*Salix miyabeana*).

One 0.6 ha (5972.4 m^2) willow plantation was established in the May 2007. It is located in the University of Saskatchewan Horticulture Field Laboratory (52^o 7' 35.91" N, 106^o 36' 26.43" W) in Saskatoon. This site is situated within the city limits on agricultural land in the Prairie Ecozone of Saskatchewan. It is a flat landscape that lies in the Saskatoon Plain region and is overlain by glacio-lacustrine material (Sutherland association). It is part of the dark brown soil zone of Saskatchewan and is dominated by heavy clay soil type, and is classified as an Orthic Vertisol. Canada thistle (*Cirsium arvense*) and redroot pigweed (*Amaranthus retroflexus*) are among the primary weed types found in the area. All 30 willow clones received from SUNY-ESF were planted here, although only the six mentioned above were used in this experiment. The soil bulk density at this site is 1.28 g cm^{-3}.

The 0.1 ha plantation in Estevan is located 10 km southeast of the town (49^o 4' 37.03" N, 102^o 52' 36.11" W). This site was prepared in May and June 2007, and planted on June 5, 2007. The soil type in the area is clay to loam, and are classified as Orthic Regosol. The bulk density of the soil at this site is 1.30 g cm^{-3}. Quackgrass (*Agropyron repens*), lamb's-quarters (*Chenopodium album*) and Canada thistle are among the common weed types found here.

The 0.1 ha plantation near Prince Albert is located at the Pacific Regeneration Technologies Inc. nursery (53^o 21' 19.39" N, 105^o 46' 25.80" W) north of the city. The site was prepared and planted on June 1, 2007. The dominant soil type here is sandy to loamy sand, and is classified as an Orthic Eutric Brunisol. The soil bulk density at this site is 1.58 g cm^{-3}. The weed population at this site is minimal, with the exception of a few interspersed dandelions (*Taraxacum officinale*).

The 0.1 ha Birch Hills plantation is located 6 km northwest (53^{o} 0' 7.08" N, 105^{o} 29' 22.72" W) of the town. The site was prepared and planted in June 2007. The soil type in this region is silt loam to clay loam, and is classified as Orthic Black Chernozem. The soil bulk density is 1.03 g cm^{-3}. This plantation has a considerable weed population despite herbicide application and sporadic mechanical weeding. Common weed types here included Canada thistle and volunteer canola (*Brassica napus*) from the previous year's crop. All sites and their associated soil and site characteristics are listed in Table 4.1.

4.2.2 Experimental design and plantation establishment

The experiment was established as a factorial design with two treatments - site and clone, being applied to determine a relationship between the treatments. As the clone treatment is applied within site, it is said to be a split plot design. Each site is arranged in a randomized block design. The clones were randomly distributed within each block at each site with the clones arranged in 6.3 x 7.9 m plots. Each plot consisted of three double-rows with thirteen trees per row. The cuttings in the rows were spaced 60 cm between and within the row and each double-row was spaced 1.5 m apart. The outer rows in each plot acted as a buffer, while cuttings in the centre double-row were used as the measurement plants. The unrooted cuttings were 25 cm long, with diameters ranging from 8-21 mm and planted in June 2007. They were kept at –4 ºC until planting. All sites were mechanically prepared by deep tilling using a tandem disc and a Case IH 165 H.P. tractor and chemically treated with the pre-emergent herbicide oxyflurofen (Goal®) (Rohm and Haas Co., Philadelphia, PA) at a rate of 2 L ha^{-1} immediately after planting using a BX2350 Kubota tractor and an 5.5 m boom sprayer. Additional herbicide treatments were applied in Birch Hills, Estevan and Saskatoon with glyphosate (Roundup-Weathermax®) (Monsanto Co., St. Louis, MO) at a rate of 2 L ha^{-1}. The Prince Albert site did not receive this additional herbicide treatment because the weed population was of little concern compared to the other three sites. Saskatoon received a supplementary herbicide treatment of bromoxynil (Pardner®) (Rhone-Poulenc, Inc., Monmouth Junction, NJ; Union Carbide Agr. Products Co., Inc., Jacksonville, FL) at a rate of 0.5 L ha^{-1} in July 2007. All sites were coppiced in April 2008 and sprayed with the herbicide Simazine 480 (Princep®) (Ciba-Geigy Corp., Greensboro, NC) at a rate of 7 L ha^{-1}, with the exception of the Prince Albert plantation, which was sprayed at a rate of 4.7 L ha^{-1}.

Table 4.1 Soil and site characteristics and weed control of four willow plantation trial sites located throughout Saskatchewan.

Site	Site and Soil Characteristics				Weed Control			
	Association	Soil Type	Soil Texture	Mean Annual Precipitation † (mm)	Pre-emergent		Post-emergent	
					Mechanical	Chemical	Mechanical	Chemical
Prince Albert *	Pine	sand to loamy sand	Orthic Eutric Brunisol	406	Deep Till	Goal® (2 L ha^{-1})	Tillage and hand weeding	Simazine 480 (4.7 L ha^{-1})
Birch Hills ‡	Hoey-Blaine Lake	silt loam to clay loam	Orthic Black Chernozem	406	Deep Till	Goal® (2 L ha^{-1})	Tillage and hand weeding	Glyphosate (2 L ha^{-1}) Simazine 480 (7 L ha^{-1})
Saskatoon §	Sutherland	heavy clay	Orthic Vertisol	360	Deep Till	Goal® (2 L ha^{-1})	Tillage and hand weeding	Glyphosate (2 L ha^{-1}) Bromoxynil (0.5 L ha^{-1}) Simazine 480 (7 L ha^{-1})
Estevan ††	Alluvium	clay loam	Orthic Regosol	418	Deep Till	Goal® (2 L ha^{-1})	Tillage and hand weeding	Glyphosate (2 L ha^{-1}) Simazine 480 (7 L ha^{-1})

† Values based on 30 year average (Fung et al., 1999).
* For a complete description see SCSR (1976).
‡ For a complete description see SCSR (1989).
§ For a complete description see SCSR (1978).
†† For a complete description see SCSR (1997).

4.2.3 Sequential soil coring

Roots were collected in September 2007, June 2008 and September 2008 using the sequential soil coring method (Milchunas, 2009; Rytter and Hansson, 1996). Core samples were extracted from all six willow clones at all four sites to a depth of 30 cm with an 8 cm diameter bucket auger. The two samples that were collected from within the measurement tree rows within each plot were spatially dispersed in order to ensure an accurate representation of the sample population. At each sampling time, 48 cores were collected per site (2 samples x 6 clones x 4 replicates). Upon extraction, the samples were bagged, labeled and transported to cold storage where they were kept at 4 º C until processing.

The root samples were processed by placing them in a mesh screen (0.5 mm mesh size) and rinsing the soil from the roots under running water. Fine roots were defined as those less than 2 mm in diameter for this analysis. The samples were dried for a minimum of 24 hours at 60˚C and weighed to obtain root biomass values, expressed in Mg ha^{-1}. The fine roots are presented on an area basis, but to a depth of 30 cm.

4.2.4 Whole tree excavation method

In July 2009, whole tree extractions were carried out to examine the coarse root structure and relative biomass of the entire plant aboveground and belowground systems. One plant of each of the six clones was selected from Saskatoon and Prince Albert, as these two sites represent the widest range in soil texture. The trees were measured for height and root collar diameter 30 cm from the base. They were then defoliated and stems were cut off at the base. Using a shovel and soil knives, the root system was removed from the soil within a 1 m radius from the cutting to a depth of approximately 30 cm with minimal damage to the structure of the roots and the remaining vegetation. The samples were stored at 4ºC until processing. Upon processing, the length of lateral roots was measured. Lateral roots' lengths were measured from the cutting and any that extended beyond 30 cm were cut off at 30 cm. Roots, stems and leaves were placed in the drying room, and were dried at 40˚C for a period of two weeks and weighed. Root biomass is presented on an area basis of Mg ha^{-1}, to a depth of 30 cm.

4.2.5 Carbon storage

Carbon content of the fine root portion of the plant system was determined from roots collected using the soil core method (section 4.2.3). Roots were ground and oven dried over night at 60˚C. Four samples per clone for each site were analyzed on the C632 LECO Carbon Determinator

(Leco Corporation, 2006). These percentages were used in conjunction with biomass values obtained from the samples to estimate the amount of C being stored in the fine root system, and to determine differences between the clones and/or sites.

4.2.6 Statistical analysis

Analysis of the soil coring fine root biomass values was carried out on the biomass using a linear mixed-effects model in R (Hornik, 2008). This model was chosen due to its explanatory power regarding repeated measures. The values displayed a non-normal distribution, thus they were square root transformed for the analysis, but presented as untransformed data. Due to the categorical nature of the explanatory variables, a regression analysis was not fit to display clear trends or relationships in the data. Model selection was performed on the data by creating a maximal model and successively excluding interacting and single terms until the model could not be further simplified. Statistical analysis was done using a 95% confidence interval ($P < 0.05$).

4.3 Results

4.3.1 Sequential soil coring biomass estimates

The Prince Albert site had significantly greater biomass when compared to the other three sites at all three sampling times (Table 4.2). Fine root biomass ranged from 0.022 Mg ha^{-1} in Saskatoon in fall 2007 to 0.915 Mg ha^{-1} in Prince Albert in fall 2008. Figure 4.1 displays the root biomass (Mg ha^{-1}) of each clone at each site, and for each sample time. When performing model selection in R to determine the best model fit to the data, there was no significant interaction between site and sample time, and there was no significant relationship between fine root biomass and clone (Table 4.3). Fine root biomass was significantly different between each site, with the exception of Saskatoon and Estevan. Fine root biomass significantly changed between each sample time. The box plots displaying the distribution of the square root transformed fine root weights against site and sample time are found in Appendix E.

Table 4.2 Mean fine root biomass (n = 24) of all clones at each site at each sampling time (values in parentheses indicate standard deviation).

Site	Fine Root Biomass			
	Fall 2007	Spring 2008	Fall 2008	Average
	----------Mg ha^{-1}----------			
Prince Albert	0.260 (0.090)	0.270 (0.099)	0.915 (0.482)	0.483 a †
Birch Hills	0.051 (0.050)	0.094 (0.033)	0.723 (0.138)	0.289 b
Saskatoon	0.022 (0.015)	0.101 (0.021)	0.298 (0.091)	0.140 c
Estevan	0.035 (0.021)	0.147 (0.064)	0.387 (0.219)	0.190 c
Average	0.092 a ‡	0.153 b	0.581 c	

† For each site, means followed by the same letter in a column are not significantly different ($P < 0.05$) using model selection in R.

‡ For each time, means followed by the same letter in a row are not significantly different ($P < 0.05$) using model selection in R.

4.3.2 Soil excavation biomass estimates

Prince Albert had the highest coarse root biomass for clones Canastota, Sherburne, SX61, and SX64, while Saskatoon had the greatest biomass for Fish Creek and Allegany (Figure 4.2). Root biomass values for the Prince Albert site ranged from at 0.128 to 1.782 Mg ha^{-1}, for clones Allegany and SX64, respectively. Average biomass for all the clones at each site was 0.973 Mg ha^{-1} in Prince Albert, and 0.493 Mg ha^{-1} in Saskatoon. In Prince Albert, most of the clones tended to have thicker, more extensive lateral root systems. The clone Canastota in Prince Albert had the longest lateral root, reaching 128 cm from the cutting. Table 4.4 includes all the aboveground and belowground data for the whole tree excavations, as well as a root: shoot ratio. Images of the excavated root systems can be found in Appendix F.

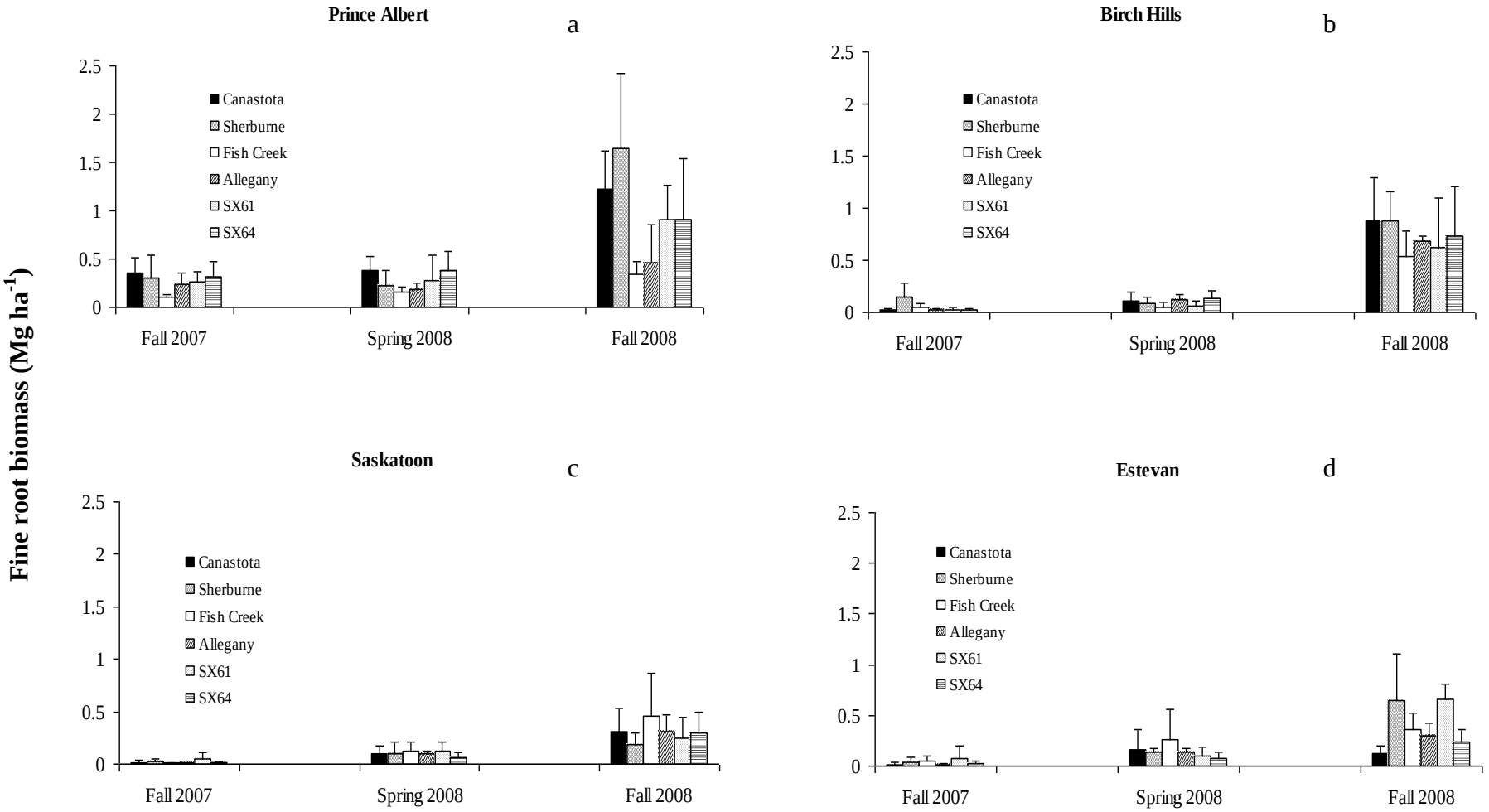

Figure 4.1 Mean fine root biomass (n = 4) for each clone at the Prince Albert (a), Birch Hills (b), Saskatoon (c), and Estevan (d) sites at each sample time using the soil coring technique. There was no significant difference between clones, and no site by time interaction ($P < 0.05$). Vertical bars represent one standard deviation.

Table 4.3 An ANOVA table of the simplified linear mixed-effects model containing the two significant explanatory variables.

	Numerator DF	Denominator DF	F-value	P-value
Site	3	12	19.7410	0.0001
Time	2	270	166.1265	< 0.0001

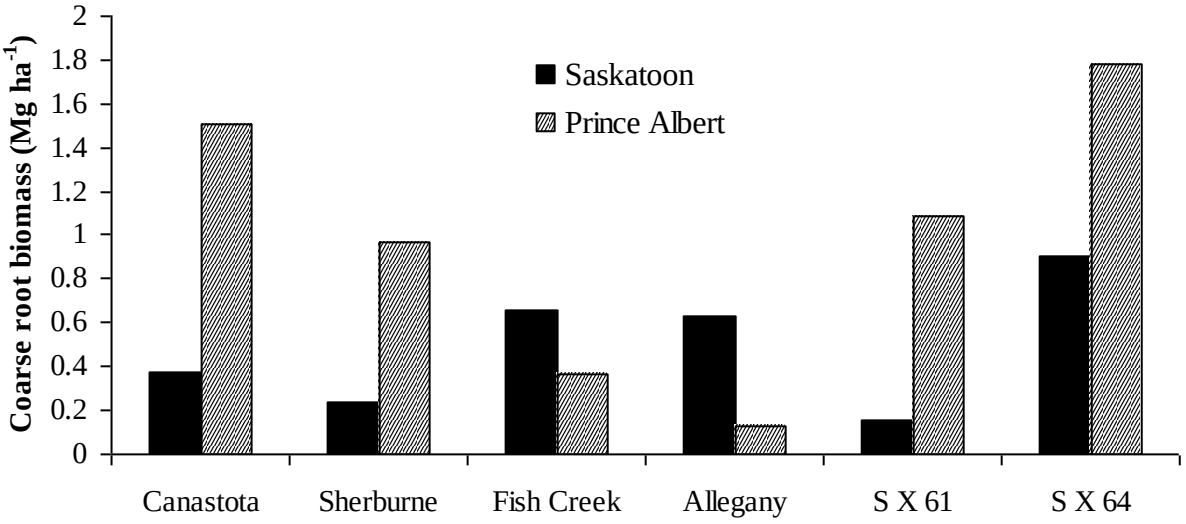

Figure 4.2 Coarse root biomass (n = 1) without the cutting of each clone in Saskatoon and Prince Albert using the whole tree excavation method.

4.3.3 Sequestration of carbon in fine willow roots

Carbon concentration of fine willow roots ranged from 40.5 % in the SX61 clone at Saskatoon to 48.1 % for the Sherburne clone in Prince Albert. The average C concentration for all the willow roots combined was 44.6 %. The % C concentration for the individual clones was applied to the root biomass values to calculate C accumulation captured in the root system from the soil coring technique. Carbon concentration did not significantly differ among the clones or sites.

Figure 4.3 depicts a similar pattern among the four sites and six clones as was seen in fine root biomass. The only significant variation in fine root C storage is seen in site and time ($P < 0.05$). All sites are significantly different from one another with the exception of Saskatoon and Estevan. There was no significant interaction between site and time. All three sample periods were significantly different from each other. Throughout all sample periods, the average values for all the clones at each site ranged from 0.010 to 0.426 Mg C ha^{-1}. Table 4.5 shows the average Mg C ha^{-1} value for all the clones at each site for

Table 4.4 Aboveground and belowground data for each clone at each site in July 2009 obtained using the whole tree excavation method.

		Aboveground						Belowground			
Site	Clone	Height (cm)	Root Collar Diameter (mm)	Number of Stems	Stem Biomass (g)	Leaf Biomass (g)	Total Aboveground Biomass (g)	Root Biomass With Cutting (g)	Root Biomass Beyond 30 cm (g)	Total Belowground Biomass (g)	Root: Shoot Ratio
Saskatoon	Canastota	150.0	13.0	3	112.0	58.2	170.2	40.5	1.6	42.0	0.25
	Sherburne	116.5	11.9	2	65.6	34.5	100.1	21.3	1.0	22.3	0.22
	Fish Creek	167.8	12.9	3	181.9	67.5	249.4	37.2	3.1	40.3	0.16
	Allegany	109.2	9.6	5	120.6	62.5	183.1	32.64	3.0	35.6	0.19
	SX61	98.5	11.4	5	40.7	28.5	69.2	12.3	0.7	13.0	0.19
	SX64	194.2	14.9	5	256.0	143.6	399.6	61.6	9.7	71.2	0.18
	Average	139.4	12.3	3.8	129.5	65.8	195.3	34.3	3.2	37.4	0.20
Prince Albert	Canastota	182.4	11.0	11	456.4	163.2	619.6	87.2	12.4	99.5	0.16
	Sherburne	230.7	11.5	5	209.6	64.3	273.9	64.1	5.1	69.2	0.25
	Fish Creek	137.0	17.2	8	167.0	48.3	215.3	28.5	2.3	30.9	0.14
	Allegany	65.0	4.4	10	16.2	9.8	26.0	13.2	0.0	13.2	0.51
	SX61	165.3	10.0	10	324.8	128.2	453.0	32.2	8.7	41.0	0.09
	SX64	188.0	10.7	12	369.0	128.0	497.0	87.3	15.2	102.5	0.21
	Average	161.4	9.1	9.3	257.2	90.3	347.5	52.1	7.3	59.4	0.23

each sample time. Prince Albert had the highest root C sequestration for all three sample periods. Each site increased in root C sequestration through each successive sample period.

Figure 4.3 depicts a similar pattern among the four sites and six clones as was seen in fine root biomass. The only significant variation in fine root C storage is seen in site and time ($P < 0.05$). All sites are significantly different from one another with the exception of Saskatoon and Estevan. There was no significant interaction between site and time. All three sample periods were significantly different from each other. Throughout all sample periods, the average values for all the clones at each site ranged from 0.010 to 0.426 Mg C ha^{-1}. Table 4.5 shows the average Mg C ha^{-1} value for all the clones at each site for each sample time. Prince Albert had the highest root C sequestration for all three sample periods. Each site increased in root C sequestration through each successive sample period.

4.4 Discussion

4.4.1 Sequential soil coring fine root biomass

The fine root biomass values found in this study compare to those found in a four-year-old willow plantation in Sweden grown on a heavy clay soil, which ranged from 0.83 to 1.7 Mg ha^{-1} (Rytter, 1999). Although the biomass values found in this study fall slightly outside the lower end of the range recorded by Rytter (1999), Prince Albert falls within the range in Fall 2008, with biomass values of 0.915 Mg ha^{-1}. The differences in fine root biomass among sites are thought in part to be a response of the various soil textures at each of the sites. The soil type at the Prince Albert site was a sandy to loamy-sand texture which suggests the roots could easily penetrate, whereas the soil types at all the other sites contained clay; a matrix through which roots may have difficulty penetrating. A study in the United Kingdom revealed more development of poplar roots in the sandy soils than other soil types, which the authors attributed to the high adaptability of poplar roots in drier environments (Crow and Houston, 2004). The lower fine root biomass at the Saskatoon plantation is possibly due to the mechanical restrictions of vertical root growth in clay type soils, and therefore the inability to access an adequate amount of water (DesRochers and Tremblay, 2009). Low historical precipitation (Table 4.1) accompanied by high air temperatures might suggest that drought conditions in Saskatchewan inhibit root proliferation through a

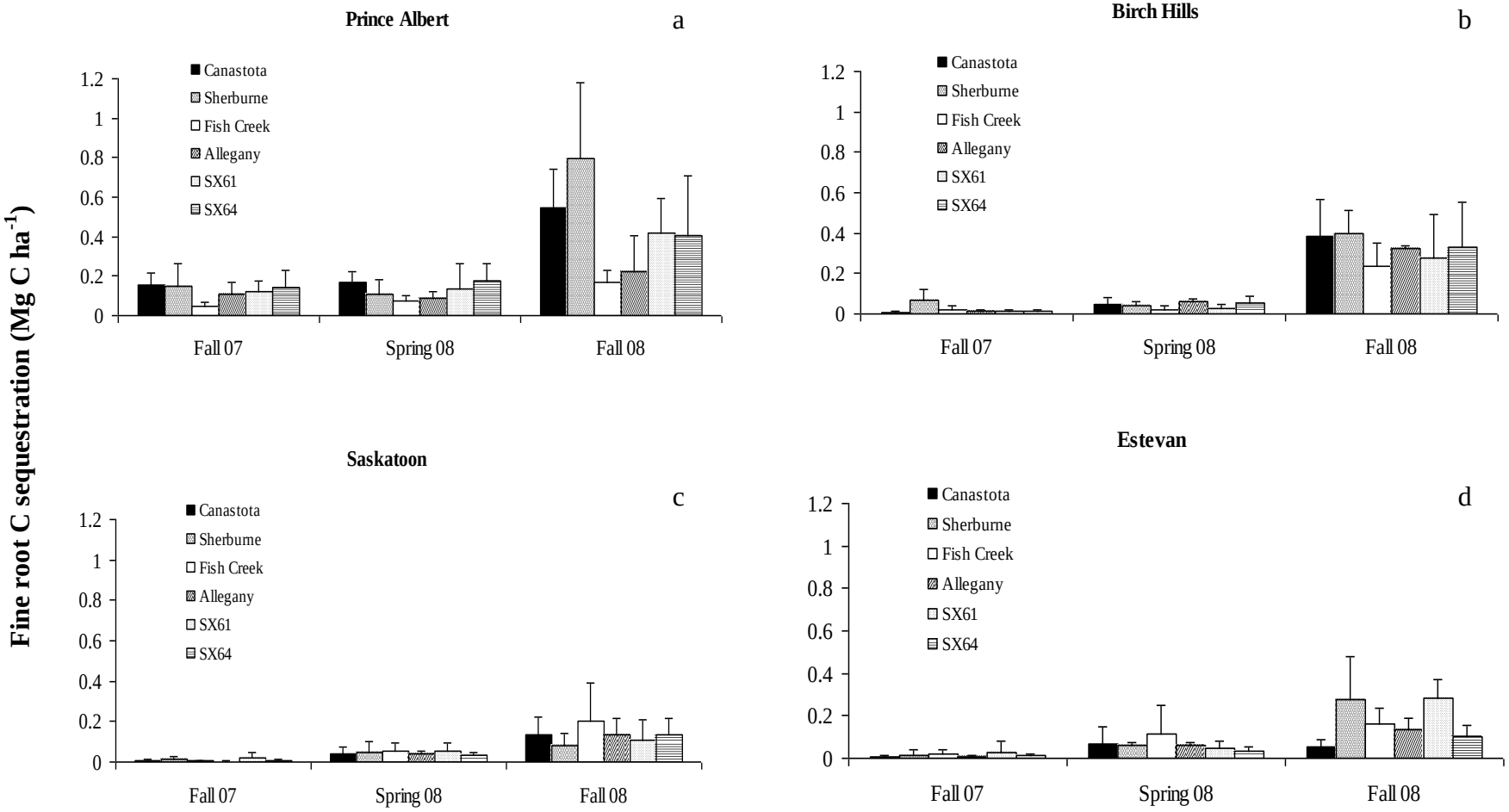

Figure 4.3 Mean fine root C sequestration (n = 4) at each sampling time for the Prince Albert (a), Birch Hills (b), Saskatoon (c), and Estevan (d) sites. There was no significant difference between clones, and no site by time interaction ($P < 0.05$). Vertical bars indicate one standard deviation.

Table 4.5 Mean root carbon content (n = 24) of all clones at each site at each sampling time (values in parenthesis indicate standard deviation). Clone was not considered a significant variable in root C content. Means site values are significantly different from each other with the exception of Saskatoon and Estevan using model selection in R ($P < 0.05$).

Site	Fine Root C Sequestration			
	Fall 2007	Spring 2008	Fall 2008	Average
	---------- Mg C ha^{-1} ----------			
Prince Albert	0.120 (0.040)	0.124 (0.042)	0.426 (0.229)	0.223 a †
Birch Hills	0.023 (0.023)	0.042 (0.015)	0.325 (0.063)	0.130 b
Saskatoon	0.010 (0.007)	0.045 (0.009)	0.133 (0.041)	0.062 c
Estevan	0.015 (0.009)	0.065 (0.029)	0.169 (0.094)	0.083 c
Average	0.042 a ‡	0.069 b	0.263 c	

† For each site, means followed by the same letter in a column are not significantly different ($P < 0.05$) using model selection in R.

‡ For each time, means followed by the same letter in a row are not significantly different ($P < 0.05$) using model selection in R.

clay soil texture. Table 4.6 is the weather station data for total monthly precipitation at each site from July to September 2008. These data indicates that Estevan received the greatest amount of precipitation, while Prince Albert received the lowest. However, the tipping bucket in the rainfall sampler in Prince Albert was frequently plugged with debris, causing potential problems with the data. Weed competition at the Birch Hills, Saskatoon and Estevan sites may have also impacted willow root growth, as additional competition for water and nutrients was likely a factor at these sites.

Table 4.6 Total monthly precipitation (mm) at each site from May to September 2008.

	Total Monthly Precipitation (mm)					
	May	June	July	Aug	Sept	Average
Site						
Prince Albert	9.8	30.7	48.0	25.4	11.1	25
Birch Hills	9.1	30.2	95.5	27.5	25.3	37.5
Saskatoon	3.4	56.7	66.0	29.0	13.7	33.8
Estevan	49.2	53.6	39.6	64.1	71.6	55.6

Soil coring root biomass estimates in this study were smaller than the minirhizotron biomass estimates discussed in Chapter 3 of this thesis. In June 2008, the average minirhizotron fine root biomass in Saskatoon was 1.41 Mg ha^{-1}, which is almost 14 times greater than the average soil coring fine root biomass in Saskatoon in June 2008. This discrepancy could be a result of the tendency of the minirhizotron method to overestimate fine root biomass in a Vertisolic soil due to

tube movement (refer to section 3.4.1), or mechanical difficulties in the soil coring method in removing the cores from a clay soil.

4.4.2. Excavation root biomass

Although whole tree excavations are generally not suitable for fine root examination, because many of the fine roots are lost (Friend et al., 1991; Uri et al., 2002), we were able to investigate the structural root system of willow rooting system at the Prince Albert and Saskatoon sites. The root: shoot ratio for all the clones at both sites ranged from 0.09 to 0.51. This range is similar to values found in a hybrid poplar system in India, which range from 0.12 to 0.31 (Swamy et al., 2006). Guidi and Labrecque (2010) determined willow root: aboveground biomass ratio to be 0.54 in an irrigated potted trial. Most of the clones had more successful root establishment at the Prince Albert site than the Saskatoon site, as the soil texture allowed for easier root penetration. Allegany and Fish Creek were, however, less successful at root establishment at the Prince Albert site than the Saskatoon site. One possible explanation for this may be related to the effect that shoot pruning has on root development. In a study on the effects of pruning on the success of hybrid poplar clones in northwestern Quebec, DesRochers and Tremblay (2009) found less extensive establishment of root systems under pruned trees versus unpruned trees. They suggested that this was an attempt of the plant to re-equilibrate nutrient and water transpiration with absorption. The aboveground portion of the clone Allegany at the Prince Albert site was the only clone to be extensively damaged due to deer browsing. The substantially lower root biomass here compared to the same clone at the Saskatoon site could be a direct effect of deer browsing. It is unclear why there is lower root biomass in the clone Fish Creek in Prince Albert than in Saskatoon, because this clone was not extensively browsed in Prince Albert. The clonal average of root biomass at the Saskatoon site determined with the soil excavation method in July 2009 (0.493 Mg ha^{-1}) was greater than the fine root biomass determined with the soil coring method at the Saskatoon site in September 2008 (0.298 Mg ha^{-1}). This was to be expected, as they sampled different portions of the root population. At the Prince Albert site, a clonal average of the root biomass determined with the soil excavation method in July 2009 (0.973 Mg ha^{-1}) was similar to the fine root biomass determined with the soil coring method in September 2008 (0.915 Mg ha^{-1}). At this site, both methods were executed with less mechanical difficulty, as it was a loose textured soil. Root biomass at the Saskatoon site was slightly less than root biomass in an irrigated potted trial in Quebec, in which 0.63 Mg ha^{-1} were recorded (Guidi and Lebrecque, 2010), while root biomass at the Prince Albert site is slightly greater.

4.4.3 Carbon sequestration of willow fine roots

The potential for soil carbon storage is one of the primary benefits of establishing a short rotation woody plantation on marginal or degraded land. As belowground biomass accumulates, more carbon is incorporated into the soil. The contribution of the roots to the soil CO_2 efflux is proportional to fine root production (Norby et al., 2002). Our average value for carbon content of willow roots is 45%, which is comparable to 42% found by Girouard et al. (1999) in a short rotation willow system in eastern Canada. As the roots senesce, the less recalcitrant portions decompose and are incorporated into soil organic matter. This portion is dependent on factors such as climate, edaphic conditions and fine root diameter (Puttsepp, 2004). Although, C sequestration under short rotation coppice is lower than under forested land, it is greater than under annual conventional crops on arable land because tillage is typically reduced (Boman and Turnbull, 1997; Richter et al., 1990). A comprehensive study of the C sequestration potential of agroforestry systems estimated the overall global land base under agroforestry systems to be approximately 1,293 million ha, and the C sequestration potential under this vast area is dependent on the climatic conditions and soil management practices (Baum et al., 2009; Nair et al., 2009). Table 4.7 gives a comparison of C sequestration of various biological components in these ecosystems. It has been suggested that plantations in the initial years following establishment sequester less carbon if the mineralization rate is initially high, or experience C loss due to tillage and site preparation practices (Girouard et al., 1999; Hansen, 1993). The estimated fine root C content of willow in this study in fall 2008 ranged from 0.133 Mg C ha^{-1} in Saskatoon to 0.426 Mg C ha^{-1} in Prince Albert. These values are smaller than those found by Zan et al. (2001) in a study on a 4 year old plantation in southwestern Quebec, which estimated willow fine root C to be 2.3 Mg C ha^{-1}. This could be due to the differences in stand age between the plantations in study and the one in Quebec, among other climatic factors.

Table 4.7 Carbon content of individual components of vegetation in arable land, short rotation coppice, and forest settings. Adapted from Boman and Turnbull (1997).

Component	Arable land	Short rotation coppice	Forest
	----------Mg C ha^{-1} ----------		
Leaves	4.0	2.5	2.5
Trunks	0	21.0	70.0
Weeds	0.4	1.0	2.0
Litter	0.4	5.0	15.0
Roots	**2.0**	**5.5**	**10.0**
Soil	25.0	35.0	45.0
Total	32.0	69.5	144.0

4.5 Conclusion

Fine root systems under short rotation willow coppice in Saskatchewan appear to be more responsive to edaphic controls on the plant system than to inherent biological characteristics of the plant. It is known that soil type and moisture regime have an important relationship with the growth response of the vegetation community (Wikberg and Ogren, 2007; Hancock et al., 2008). In this study, it is likely that soil texture and moisture were limiting factors on willow fine root growth in Saskatchewan. The biological aspect of the study, involving the six willow clones, had no appreciable influence on fine root growth.

In assessing the influence of soil type on rooting habits of willow in Saskatchewan, it was observed that the sandy soils provided better growth environments than heavy clay soils for willow roots. Willow is widely understood to be a plant variety that is dependent on large quantities of water. In the relatively dry climate of Saskatchewan, the historical mean annual precipitation is lower than in other Canadian provinces. In areas of insufficient moisture, roots are likely to extend laterally and vertically to locate sources of water. However, dry, fine textured soils provide mechanical restrictions to root growth that are comparatively of less concern in coarse textured soils. Inability of the willow roots to reach a source of water will result in poorly developed aboveground and belowground biomass. It is suspected that the lack of soil moisture at some sites had a substantial effect on willow fine root growth. However, further studies examining the relationship between soil moisture and willow root growth are needed before inferences are made in this regard.

Our study indicated that although there was no significant interaction between clone and fine root biomass or amount of C sequestered, there was a site effect on fine root biomass and C sequestered. Therefore we would reject the null hypothesis. The importance of soil type and

available moisture on the establishment of willow root systems is highly appreciated in dry clay soil types found in much of southern Saskatchewan. Future development of the root system as the plantation ages may actuate greater underground development of the plantation, therefore adding to the soil C sequestration potential, and greater development of the marketable aboveground portion of the crop.

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5.0 GENERAL DISCUSSION AND CONCLUSIONS

Climate change has confounded and motivated scientists and policy makers, resulting in alternative energy sources being explored for the future health and vitality of the planet. Along with solar and wind, biomass is one challenge in the attempt to identify renewable and reliable sources of alternative energy. Various research trials have identified the growth and phytoremediation potential of short rotation willow crops, and concerted efforts have been organized to promote the development of willow biomass plantations for bioenergy. The Salix Consortium is one such effort that was intended to turn willow crops from experimental trials into a commercial business enterprise in the United States, and is based on research from the United States (Volk et al., 2006), Sweden (Mola-Yudego and Gonzales-Olabarria, 2010), Canada (Mosseler, 1990), and the United Kingdom (Armstrong et al., 1999; Bell et al., 2006), with aboveground yields reaching as high as 70.4 Mg ha^{-1} (Labrecque and Teodorescu, 2003). In Canada, governmental programs such as the Agricultural Bioproducts Innovation Program (ABIP) and the Saskatchewan Biofuels Investment Opportunity (SaskBIO) have been developed to promote the biofuels industry in Canada, and encourage the transfer of information between researchers and landowners. As a majority of the literature focuses on the aboveground portion of willow bioenergy plantations, there is much to be discovered about the belowground portion in terms of its ability to establish extensive root systems in various environmental conditions, and the relationship between belowground and aboveground yield.

The aim of this study was to examine willow roots systems grown in an intensive short rotation woody crop in Saskatchewan and determine the C sequestration potential of the system. As this NSERC project is the first attempt to establish a willow energy system in Saskatchewan, the results of this study will contribute to an information system for the development of intensive short rotation woody crops in this region.

The two methods used to examine root growth have highlighted both their advantages and limitations in providing data for a wide spectrum of root parameters. Destructive sampling, such as soil coring, can not provide accurate answers in the longevity and turnover of a fine root system due to the infrequent sampling intervals, although this method is still widely used in root productivity studies (Milchunas, 2009). The minirhizotron method, although better suited to examine the temporal dynamics of the fine root population, may result in the overestimation of fine root biomass, because experimental artifacts such as soil-tube interface gaps may misrepresent conditions in the bulk soil for certain soil types such as Vertisols.

Statistical testing showed that there was no significant clone effect on fine root biomass, but there was a significant site effect, indicating a greater degree of environmental rather than biological influence over willow root growth. Perhaps the differences in fine root growth at each site are due, in part, to a combination of soil moisture regimes and various soil textures at each site. It is suggested that the soil texture at the Prince Albert site was a matrix through which the roots could easily penetrate to access water. This was reflected in the appreciable aboveground biomass at this site. The soil texture at the Estevan site was clay loam; at the Saskatoon site was silty clay loam to silty loam; and at the Birch Hills site was silty loam to clay loam. It is suggested that these fine soil textures impeded fine root proliferation and lateral root growth in these three sites, restricting an adequate water supply to the willow at these sites.

Fine root biomass values collected in Saskatoon using the soil coring method ranged from 0.02 Mg ha^{-1} in September 2007 to 0.30 Mg ha^{-1} in September 2008. These values are considerably smaller than those found using the minirhizotron method, which ranged from 0.78 Mg ha^{-1} in May 2008 to 7.39 Mg ha^{-1} in September 2008, and reached as high as 25.75 Mg ha^{-1} in September 2009. The average root biomass value in Saskatoon determined using the excavation method was recorded at 0.493 Mg ha^{-1}.

The minirhizotron is able to provide estimates in root turnover and productivity, but the accuracy of the results is highly dependent on the soil-tube contact in the soil. Therefore, it is not recommended that the minirhizotron method be used in soils with a high degree of shrinking/swelling. The tubes in this study were installed in a Vertisolic soil, and therefore were subjected to moving and bending by the movement of the soil with the shrinking and swelling cycles during wet and dry seasons. Other studies have examined fine root growth using the minirhizotron in a Vertisolic soil (Ito et al., 1997; Krishnamurthy et al., 1998), although these studies did not identify the limitations of using this method in a Vertisolic soil. Smit et al. (2000) identified the development of gaps between the tube and the soil, but did not attribute them to soil motion in a Vertisol. Our work suggested that the movement of the soil created large gaps between the tube surface and the soil which opened up preferential rooting pathways for the plant roots, thus greatly overestimating fine root biomass.

The C sequestration of willow crops determined using the soil coring technique in this study in September 2008 ranged from 0.133 to 0.426 Mg C ha^{-1}. These values are smaller those found by Zan et al. (2001), who determined willow fine root C sequestration to be 2.3 Mg ha^{-1}. This could be

reflected in the differences in stand age between the two plantations or soil textures, although the latter was not identified by the authors.

The information presented here is part of a comprehensive initiative to use the Carbon Budget Model of the Canadian Forest Sector (CBM-CFS3) to predict the amount of C that could be potentially sequestered by willow bioenergy plantations grown on marginal land in Saskatchewan. The model 3PG (Physiological Principles of Predicting Growth), however, was first used to predict willow belowground biomass as input to the CBM-CFS model. The predictions of root biomass from the 3PG model after three growing season was approximately 5 Mg ha^{-1} (Amichev et al., 2010). These predicted values of root biomass are more than double the values reported in this study when considering the fine and coarse root biomass measurements together. The root biomass estimates from this study, however, do provide the modellers with the first validations of belowground biomass for their simulations and will help in validating future estimates of C sequestration belowground. The relationship between soil texture and root biomass will have to be examined more closely in the model to account for the observations from this study.

This study identified some relationships between different soil types and belowground willow biomass, and highlighted the limitations associated with examining willow root dynamics in a Vertisolic soil. Further investigation into the environmental controls on the growth of short rotation willow plantations in Saskatchewan is necessary to ensure that maximum yields are achieved for the development of a bioenergy industry.

5.1 References

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APPENDIX A

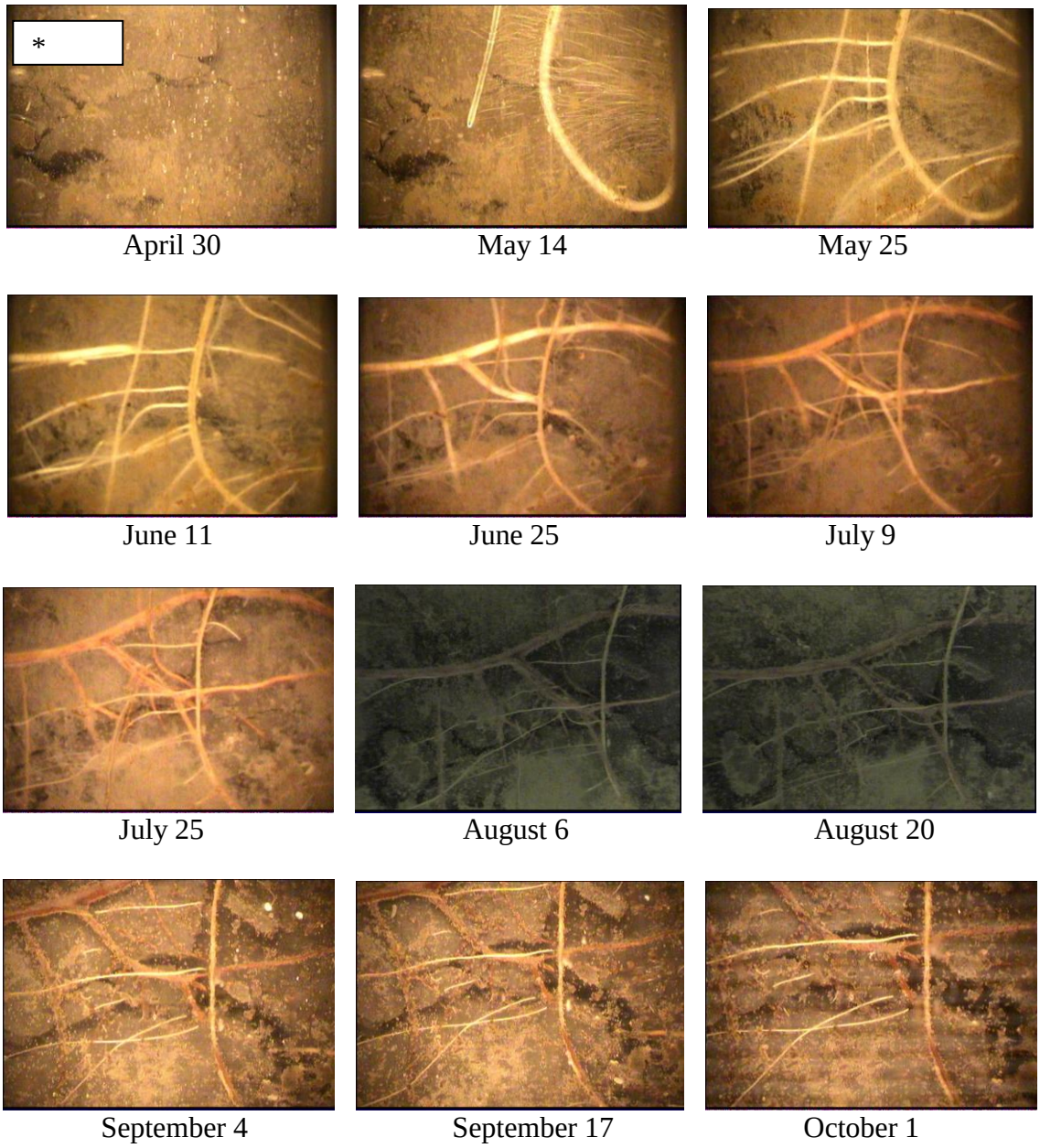


Figure A.1 Minirhizotron images taken for willow in Saskatoon from April 2008 to October 2008.
* Represents 3 mm length

APPENDIX B

```
/*This procedure is designed to process minirhizotron data in order to obtain
fine root productivity in g of dry mass per m2 of horizontal ground surface.  It
follows "Method #2"of Bernier and Robitaille, (Plant and Soil, in press as of
march 2004). For any question or comments, please contact Pierre
Bernier(pbernier@cfl.forestry.ca)

THIS VERSION IS STILL PRELIMINARY AND HAS NOT BEEN FULLY VALIDATED (2004-05-17)

Minirhizotron are transparent tubes inserted into the ground, and into which a
camera is lowered periodically to capture images of roots growing on the
outsidetube surface.  The tubes are inserted at an angle and the images are
capturedon their upper portion only. Each image is captured at a given location
or frame along the length of the tube. When analysing the images, roots are
traced on screen with an appropriate analytical software. The roots are numbered
sequentially within each frame.  Each root observation is therefore identified by
the appropriate tube number, frame number, root number and date. The only
observation needed is the diameter of that root on that date.

The first infile is the main fine root data file and is read into TMP.  Each line
must be a single observation of one particular root at one particular date. This
infile must be an EXCEL file and it must have the following column names: Tube,
Frame, Root, Date and RtDiam.

Tube, frame and root columns must contain sequential integers only(no
letters);Date must be in a Julian or day-of-year format;
RtDiam must be in mm;

The second infile contains the tube and site descriptors and is read into
TubeProp It must also be an EXCEL file and have the following column names: Tube,
TubeAngle,Slope, StonFrac, as well as parameter values for A0, A1 and A2 for
describing the specific mass of the fine roots.

Tube is the tube number as in the first infile
TubeAngle is the angle of the tube (in degrees) with respect to the ground
Slope is the slope angle (in degrees) with respect to the  horizontal
StonFrac is the fraction of coarse (D>2mm) particles in the soil (0<=stonFrac<=1)
Parameters A0, A1 and A2 describe the specific mass of the roots (in g/cm3).
A0 is an average specific mass.
A0 is given a value >0 only if an average specific mass value is used.
A0 is set to 0 if a diameter-dependant function is used to describe the specific
mass of roots
A1 and A2 are the two parameters of a Poisson function "A1*(1-exp(-A2*RootDiam)"
A1 and A2 are ajusted to field data supporting the diameter dependancy of
specific mass
A1 and A2 are set to 0 if only a mean specific mass (i.e. A0>0) is used

The computation of productivity assumes that W, the width of the minirhizotron
frames
is equal to 18mm

The results are written to an EXCEL file for which the user must provide
appropriate directory coordinates in the last procedure of this program. This
final output files produces for each observation date (except the first one) and
for each tube the following variables:  Mass_T0, Mass_T1 and productivity.
Mass_T1 is the actual mass of roots observed at the date indicated on the line.
Mass_T0 is the mass of roots seen at the previous date, but without those roots
```

```
that will have disappeared at T2 (see Bernier and Robitaille for details).
Productivity is the difference between these two numbers.  Productivity cannot be
a negative value. All masses are given in g/m2 of horizontal ground surface*/

options linesize=80;
option nolabel;

/*First infile: enter the root measurements*/
PROC IMPORT OUT= Tmp
            DATAFILE= "C:\SAS_rootinfile.xls"
            DBMS=EXCEL2000 REPLACE;
     GETNAMES=YES;
RUN;
/*Second infile: Entre the site and tube properties*/
PROC IMPORT OUT= TubeProp
            DATAFILE= "C:\SAS_tubeinfile.xls"
                  DBMS=EXCEL2000 REPLACE;
     GETNAMES=YES;
RUN;
/***************************** START DATA PREPARATION
*****************************/
/*Creates file TMP1; creates a specific ID for each observation
that is made up or the root identifiers and the  date*/
DATA Tmp1;
      SET Tmp;
      ID = COMPBL(Tube||Frame||Root);
RUN;
/*Sorts TMP1 by root ID and date*/
PROC SORT DATA = Tmp1;
      BY ID DATE;
RUN;
/*In TMP1, creates three new columns called MaxDiam ADDNEW and DECreasing*/
DATA Tmp1;
      SET Tmp1;
      MaxDiam = RtDiam;
      ADDNEW = '         ';
      DEC = '         ';
RUN;
/*Creates table DATE_ALL that contains the list observation dates*/
PROC SQL;
      CREATE TABLE DATE_ALL AS
            SELECT DISTINCT DATE
            FROM Tmp
            ORDER BY DATE;
/*Creates table Rt_FL that identifies the first and last measurement date for
each root*/
      CREATE TABLE Rt_FL AS
            SELECT ID, MIN(DATE) AS DATEF, MAX(DATE) AS DATEL
            FROM Tmp1
            GROUP BY ID
            ORDER BY ID;

/*In Rt_FL, numbers all lines sequentially in the variable "NUM"*/
DATA Rt_FL;
      SET RT_FL;
      Num = _N_;
RUN;
```

```
/*Merge Tmp1 and Rt_FL by root ID: this assigns first and last dates to all root
observations*/
PROC SQL;
      CREATE TABLE Tmp1 AS
            SELECT a.*, b.* /*b.Num*/
            FROM Tmp1 as a, Rt_fl as b
            WHERE a.ID = b.ID;
/*Counts the number of roots*/
PROC SQL;
      CREATE TABLE NBROOT AS
      SELECT COUNT(*) AS NBROOT
      FROM RT_FL;
/*Calls up macro nbroot using nbroot as input*/
DATA nbRoot;
      SET nbRoot;
      CALL symput("nbRoot",nbRoot);
RUN;
/*This macro checks root by root (unique ID) if there are missing observations
within a sequence of observations of a given root, and if diameter of that
root is decreasing.  If there are missing observations, it adds a new line
that contains the same RtDiam as the line above.  If the diameter is decreasing,
it maintains in column MaxDiam the maximum diameter ever measured for that root.
Analysis of productivity will be done using this column.  Labels "adding" and
"decrease" are added to the temporary file to identify lines that were either
added or modified.  The macro creates a new file calles "ALL" that contains
the following columns: id Date  MaxDiam ADDNEW dec.  See Bernier and Robitaille
for further explanation on the necessity of this procedure*/
%MACRO nbroot;
      /*Loop through all roots*/
      %DO i=1 %TO &nbRoot %BY 1;
            /*Add the root number to the list of dates*/
            DATA date;
                  SET Rt_fl (where = (Num = &i));
            RUN;

            PROC SQL;
                  CREATE TABLE Dates AS
                        SELECT a.*, b.*
                        FROM date AS a, date_All AS b;
            DATA Dates;
                  SET Dates;
                  IF date < datef OR date > datel THEN DELETE;
            RUN;
            DATA root;
                  SET Tmp1 (where = (Num = &i));
            RUN;
            DATA Roots;
                  MERGE Root Dates;
                  BY Date;
            RUN;

            PROC IML;
                  USE roots;
                  READ ALL;
                  matSize = nRow(MaxDiam);
                  row = 1;
                  DO WHILE (row < matSize);
                        MaxDiam_p = MaxDiam[row];
```

```
                        id_p = ID[row];
                        Tube_p = Tube[row];
                        row1 = row+1;
                        MaxDiam_n = MaxDiam[row1];
                        IF MaxDiam_n = . THEN DO;
                              MaxDiam[row1] = MaxDiam_p;
                              id[row1] = id_p;
                              Tube[row1] = Tube_p;
                              ADDNEW[row1] = 'adding';
                        END;
                        IF (MaxDiam_n < MaxDiam_p & MaxDiam_n > .) THEN DO;
                              MaxDiam[row1] = MaxDiam_p;
                              dec[row1] = 'decrease';
                        END;
                        row = row + 1;
                  END;
                  CREATE rep VAR[id Tube Date RtDiam MaxDiam ADDNEW dec];
                  APPEND;
                  CLOSE rep;

                  PROC APPEND BASE=All;
                  RUN;
%END;
%MEND;
%nbroot;
/***************************** END DATA PREPARATION
*****************************/

/***************************** START INCREMENT *****************************/
/*In this section, fine root productivity is computed as in the Method 2 of
Bernier and Robitaille (2004), using their equations 1, 2 and 4.  The variables
are:
rho: the specific mass, g/cm3
TubeAngle: the angle of the tube w/r to the ground , degrees
Slope: the angle of the ground with respect to the horizontal, degrees
StonFrac: the fraction of coarse material in the soil
A0 is the average specific mass (g/cm3) if only an average is used
A0 is set to 0 is values are provided for parameters A1 and A2
A1 is the first parameter of a two-parameter Poisson function
A2 is the second parameter of a two-parameter Poisson function
A1 and A2 are set to 0 if a value of A0 is provided


The computation assumes that W, the width of the minirhizotron frames
is equal to 18mm

This section computes the volume per unit area of ground for each individual root
All the roots are within file "ALL", a file created above in the macro*/

DATA Tubeprop2;
      SET Tubeprop;
      sinAlpha=sin(3.1416*(TubeAngle)/180);
      cosBeta=cos(3.1416*(Slope)/180);
RUN;

PROC SORT DATA = All;
BY Tube;
RUN;
```

```
data a;
merge all tubeprop2;
by tube;
run;

PROC SQL;
      CREATE TABLE All1 AS
            SELECT a.*, b.sinAlpha, b.cosBeta, b.StonFrac, b.A0, b.A1, b.A2
            FROM All AS a left join Tubeprop2 AS b
            ON a.Tube = b.Tube;
/* Computes the volume of each root as in eqs 1 and 2 of Bernier and Robitaille
      Make sure that the units of Rho, the specific mass, are g/cm3 and that
      of the root diameters is mm*/
DATA Tmp2;
      SET All1;
      rho = A0+A1*(1-exp(-A2*maxDiam));
      W=14.2;
      Ae=3.1416**2*(maxDiam/2)**2/sqrt(2);
      P = 2*10**6*(rho/1000)*(1-StonFrac)*Ae * sinAlpha*cosBeta/W;
RUN;
PROC DATASETS;
      DELETE ALL;
RUN;
/*Identifies the last date of measurement in the main file Tmp2 within a new
      file T2*/
PROC SQL;
      CREATE TABLE T2 AS
            SELECT a.*, b.num
            FROM  Tmp2 as a left join rt_fl as b
            ON a.ID = b.ID AND a.Date = b.Date1;
PROC SORT DATA = T2;
BY Tube;
RUN;
/*In new file T3_START, for a particular date, selects all roots that are not
            at their last date of measurement*/
PROC SQL;
      CREATE TABLE T3_START AS
            SELECT Tube, Date, sum(P) as Sum_P
            FROM  T2
            WHERE Num =.
            GROUP BY Tube, DATE
            ORDER BY Tube, DATE;
/*In new file T3_end, for a particular date, selects all roots, even those at
            their last date of measurement*/
      CREATE TABLE T3_END AS
            SELECT Tube, Date, sum(P) as Sum_P
            FROM  T2
            GROUP BY Tube, DATE
            ORDER BY Tube, DATE;
DATA T3_START;
      SET T3_START;
      ID_ = COMPBL(TUBE || DATE);
RUN;
DATA T3_END;
      SET T3_END;
      ID_ = COMPBL(TUBE || DATE);
RUN;
```

```
/*Add the volume computed from eq. 2 to T3_START, grouped by date*/
PROC SQL;
      CREATE TABLE T3_START AS
            SELECT b.Tube, b.Date, a.Sum_P
            FROM  T3_START as a RIGHT JOIN T3_END as b
            ON a.ID_ = b.ID_;
PROC SORT DATA = T3_START;
BY Tube;
RUN;
/*Adds a label number*/
DATA T3_START;
      SET T3_START;
      Num = _N_ ;
      IF Sum_P = . THEN Sum_P = 0;
RUN;
/*Labels the end dates sequentially*/
DATA T3_END;
      SET T3_END;
      Num = _N_ - 1;
      IF Sum_P = . THEN Sum_P = 0;
RUN;
/*Creates table "Diff" as the difference in volumes between dates "Start" and
      "END" and the resulting change in mass is attributed to the last date of
      the date pair in "b.date"*/
PROC SQL;
CREATE TABLE Diff AS
            SELECT b.Tube, b.Date, a.Sum_P AS Mass_t0, b.Sum_P AS Mass_t1,
Mass_t1 - Mass_t0 AS Increment
            FROM  T3_START as a , T3_END as b
            WHERE a.Num = b.Num AND a.Sum_P ne 0;

/***************************** END INCREMENT *****************************/
PROC EXPORT DATA= Diff
            OUTFILE= "C:\SAS_outfile.xls"
            DBMS=EXCEL2000 REPLACE;
RUN;
```

APPENDIX C

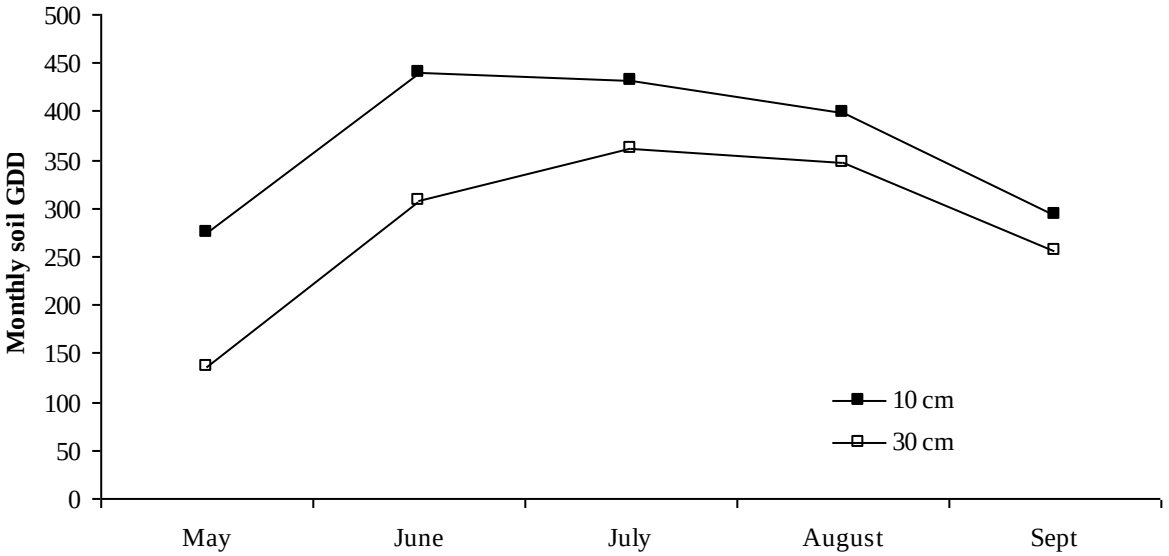


Figure C.1 Mean (n=3) monthly soil growing degree days (GDD) for all clones at 10 cm and 30 cm depth from May to September 2009 based on data collected using HOBO® H8 Temperature data loggers.

APPENDIX D

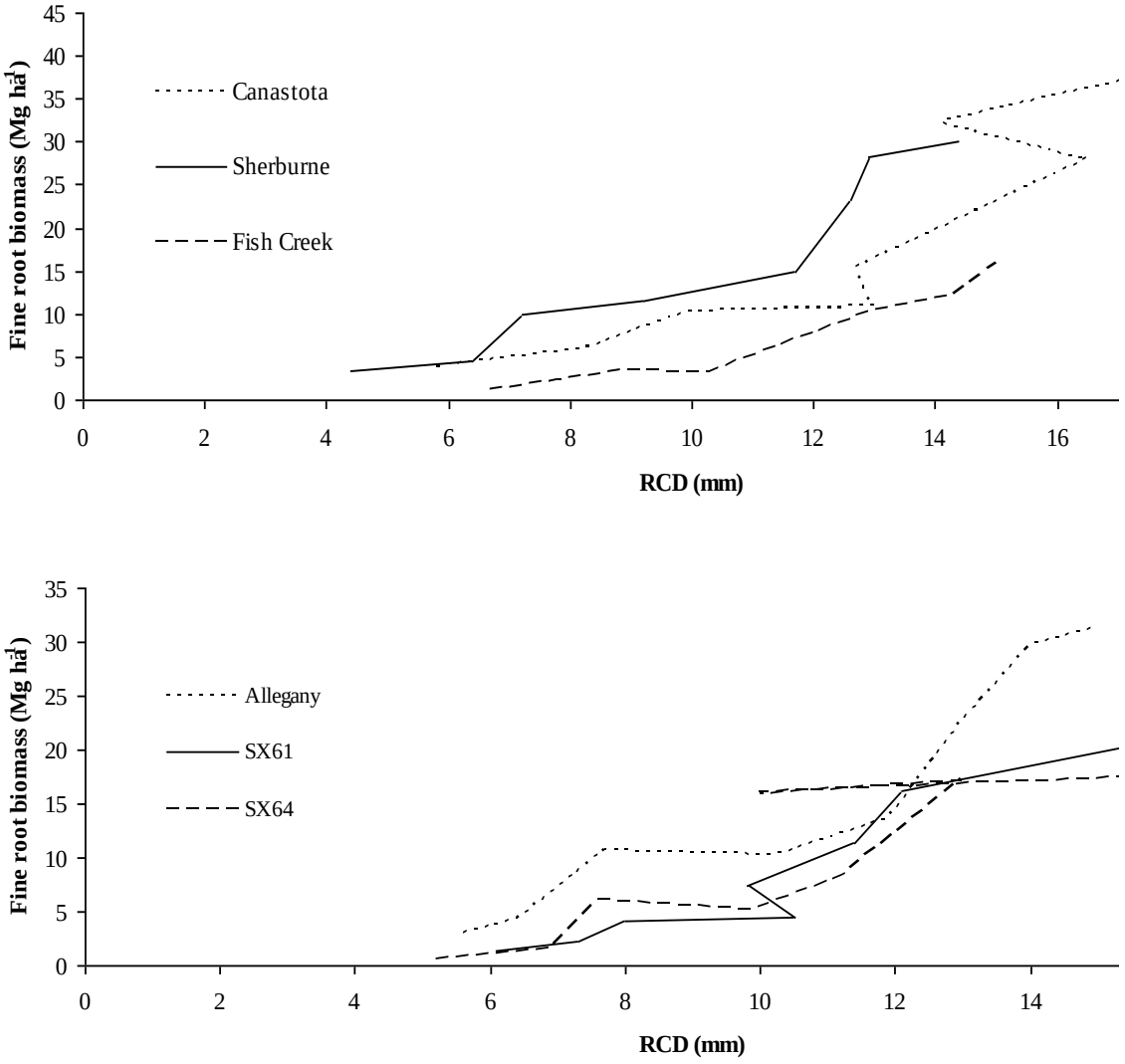


Figure D.1 The growth response between tree root collar diameter (RCD) (mm) and fine root biomass (Mg ha^{-1}).

APPENDIX E

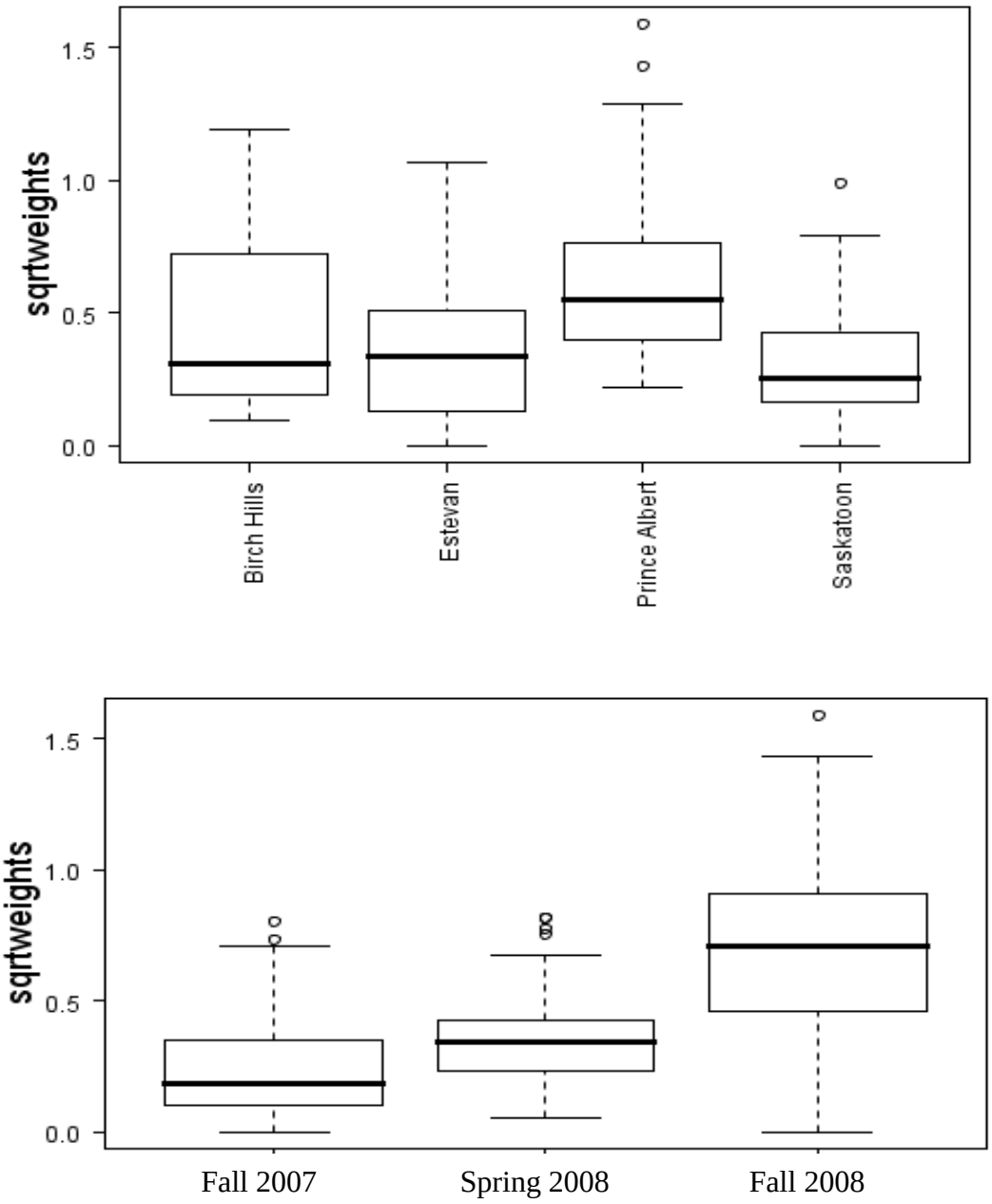


Figure E.1 Box plots depicting square root transformed weights against time and site in R statistical environment. Clone was not considered a significant explanatory variable ($P < 0.05$).

APPENDIX F

Canastota

Prince Albert

Saskatoon

Figure F.1 Images of Canastota clone excavated root system at the Saskatoon and Prince Albert sites. Note: Ruler is 30.5 cm.

APPENDIX G

Sherburne

Prince Albert

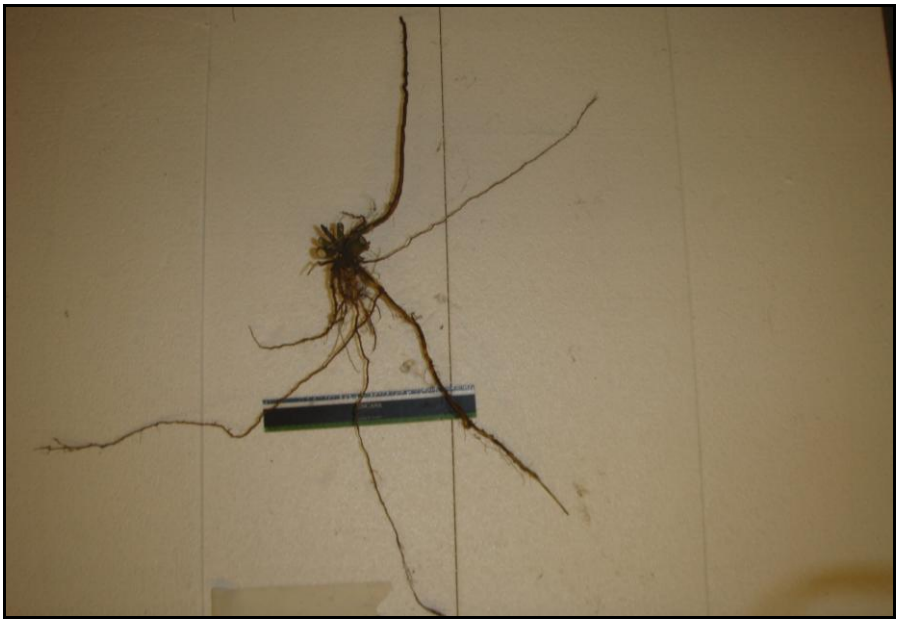
Saskatoon

Figure G.1 Images of Sherburne clone excavated root system at the Saskatoon and Prince Albert sites. Note: Ruler is 30.5 cm.

APPENDIX H

Fish Creek

Prince Albert



Saskatoon

Figure H.1 Images of Fish Creek clone excavated root system at the Saskatoon and Prince Albert sites. Note: Ruler is 30.5 cm.

APPENDIX I

Allegany

Prince Albert

Saskatoon

Figure I.1 Images of Allegany clone excavated root system at the Saskatoon and Prince Albert sites. Note: Ruler is 30.5 cm.

APPENDIX J

SX61

Prince Albert

Saskatoon

Figure J.1 Images of SX61 clone excavated root system at the Saskatoon and Prince Albert sites. Note: Ruler is 30.5 cm.

APPENDIX K

SX64

Prince Albert

Saskatoon

Figure K.1 Images of SX64 clone excavated root system at the Saskatoon and Prince Albert sites. Note: Ruler is 30.5 cm.